METHODS TO TREAT AUTOIMMUNE AND INFLAMMATORY CONDITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 60/262,849, filed January 19, 2001, the contents of which are hereby incorporated by reference into the present disclosure.

TECHNICAL FIELD

[0002] The present invention is in the field of medicinal chemistry and relates to other areas such as pharmacology and immunology. In particular, it provides methods to treat autoimmune disorders and inflammatory conditions.

BACKGROUND

[0003] The function of tumor suppressor genes is a major focus of recent attempts to develop innovative therapeutics for the treatment cancer. The products of tumor suppressor gene expression are generally characterized as negative regulators of cell proliferation (Knudson, A. G. (1993), Weinberg, R. A. (1995)). Thus, therapeutic approaches to date include gene therapies to restore inactive or missing tumor suppressor function in cancer cells to re-establish normal cellular function or induce apoptosis (Clayman, G. L. (2000), Knudson, A. G. (1993)).

[0004] Functional loss of tumor suppressor genes also has been linked to hyperproliferative inflammatory or autoimmune diseases that have cellular hyperproliferation as one of their characteristics (Cordan-Cardo, C. and Prives, C. (1999)) and/or defective apoptosis (programmed cell death) (Mountz, J. D. et al. (1994)). These include: rheumatoid arthritis, systemic lupus erythmatosus, psoriatic arthritis, reactive arthritis, Crohn's disease, ulcerative colitis and scleroderma. Table 1 lists literature examples which suggest that such a link may exist.

Table 1. Literature Examples Suggesting that Biological Expression of TP53 Tumor Suppressor Mutation/Inactivation Relates to Noncancer Hyperproliferative Disease, Autoimmune Disease and Inflammation.

Impact	*Disease Effect*	*Reference*
Increased IL6	Proliferation Inflammation Rheumatoid Arthritis	Han et al. (1999)
Increased metalloproteinases	Tissue Degradation	Sun, Y. et al. (2000)
Increased proliferation of synovial cells	Rheumatoid arthritis	Aupperle, K. R. et al. (1998)
Genetic instability and disease progression	Chronic inflammation Ulcerative colitis	Tak. P. P. et al. (2000) Lang, S. M. et al. (1999)
Increased expression of E2F regulated genes (TS, DHFR)	Proliferation Drug resistance Multiple autoimmune and inflammatory diseases	Banerjee, D. et al. (1998)
Viral proteins expression leading to p53 inactivation	Athersclerosis	Tanaka, K. et al. (1999)
Increased angiogensis	Supports hyper-proliferative States, ex. enabling atheromaorpannus formation.	Zhang, L. et al. (2000)

[0005] Loss of RB/p16 function can result in similar proinflammatory, proliferative and dedifferentiating effects on cells (Carson, R. A. and Haneji, N. (1999); Shim, J. et al. (2000); Wolff, B. and Naumann, M. (1999); DiCiommo et al. (2000)), and alteration in cell-cell interactions (Plath et al. (2000)). Inactivation of tumor suppressor function by somatic mutation or via interaction with virally-encoded proteins is proposed to contribute to the proliferative/inflammatory aspect of athersclerosis, restenosis or other hyperproliferative diseases (Tanaka, K. et al. (1999); Aoki, M. et al. (1999); Guevara, N. V. et al. (1999); Iglesias, M. et al. (1998)). Finally, the expression of the proinflammatory cytokine, macrophage inhibitory factor (MIF), may be capable of inactivating p53 function

in some cell types (Hudson, J. D. et al. (1999); Cordon-Cardo, C. and Prives, C. (1999); Portwine, C. (2000)).

DISCLOSURE OF THE INVENTION

[0006] This invention provides methods for treating cells or tissue involved in a pathology selected from the group consisting of an autoimmune disease and an inflammatory condition, by contacting the cells or tissue with an effective amount of a compound selected from the group consisting of a 1,5- substituted pyrimidine derivative or analog and furano-pyrimidone derivative or analog. The methods can be practiced in vitro, ex vivo an in vivo. In one aspect, the cells or tissue are characterized by loss of tumor suppressor function. In another aspect, the cells overexpress an endogenous intracellular enzyme such as thymidylate synthase.

[0007] When practiced in vivo in a subject, the invention provides a method for treating a subject having an autoimmune disorder or inflammatory condition by delivering to the subject an effective amount of one or more of these compounds. Methods for synthesizing the compounds are described herein and in Applicant's prior patent literature, e.g., PCT/US98/16607 and PCT/US99/01332, which describe the compounds as "ECTA" compounds or prodrugs.

[0008] Also provided herein is an assay for selecting agents that inhibit the growth of cells or tissue involved in a pathology selected from the group consisting of an autoimmune disease and an inflammatory condition.

[0009] The methods are useful to treat or ameliorate the symptoms of autoimmune diseases, for example, systemic lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, reactive arthritis, Sjögren's syndrome, graft-versus-host disease (GVHD), myasthenia gravis, atherosclerosis, glomerulonephritis, Type 1 diabetes, muscular dystrophy and osteoarthritis. The methods are also useful to treat or ameliorate the symptoms associated with an inflammatory condition, for example psoriasis, asthma, ulcerative colitis, scleroderma, inflammatory bowel disease, and Crohn's disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Figure 1 shows clinical scoring of animals with collagen-induced arthritis using NB 1011, a 5'-phosphoramidatyl deoxyuridine derivate and controls.

[0011] Figure 2 shows therapeutic effect on paw swelling in animals with collagen-induced arthritis.

[0012] Figure 3 shows histological evaluation of all joints performed by an observer blinded to the treatments received. This figure represents the percentage of joints exhibiting normal, mild or moderate to severe arthritic changes in the joint architecture in different treatment groups. Chi-square test (2 X 2 correlation) was done to calculate statistical significance of data. $P < 0.05$ (*) was considered significant.

MODES FOR CARRYING OUT THE INVENTION

General Techniques

[0013] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "MOLECULAR CLONING: A LABORATORY MANUAL" Second Edition (Sambrook et al., 1989); "OLIGONUCLEOTIDE SYNTHESIS" (M.J. Gait, ed., 1984); "ANIMAL CELL CULTURE" (R.I. Freshney, ed., 1987); the series "METHODS IN ENZYMOLOGY" (Academic Press, Inc.); "HANDBOOK OF EXPERIMENTAL IMMUNOLOGY" (D.M. Weir & C.C. Blackwell, eds.); "GENE TRANSFER VECTORS FOR MAMMALIAN CELLS" (J.M. Miller & M.P. Calos, eds., 1987); "CURRENT PROTOCOLS IN MOLECULAR BIOLOGY" (F.M. Ausubel et al., eds., 1987, and periodic updates); "PCR: THE POLYMERASE CHAIN REACTION" (Mullis et al., eds., 1994); "CURRENT PROTOCOLS IN IMMUNOLOGY" (J.E. Coligan et al., eds., 1991); and J. March, ADVANCED ORGANIC CHEMISTRY: REACTIONS, MECHANISMS AND STRUCTURE, 4th edition (John Wiley & Sons, NY (1992).

Definitions

[0014] As used herein, certain terms may have the following defined meanings.

As used in the specification and claims, the singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. Similarly, use of "a compound" for treatment or preparation of medicaments as described herein contemplates using one or more compounds of this invention for such treatment or preparation unless the context clearly dictates otherwise.

[0015] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

[0016] As used herein, the term "analog" is intended to mean a structural derivative of a compound that differs from it by at least one element. The term "derivative" is intended to mean a compound derived or obtained by another and containing the essential elements of the parent substance.

[0017] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about". It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0018] A "subject" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets.

[0019] An "effective amount" is an amount sufficient to effect beneficial or desired results. For example, a therapeutic amount is one that achieves the desired therapeutic effect. This amount may be the same or different from a prophylatically effective amount, which is an amount necessary to prevent onset of disease or disease symptoms. An effective amount can be administered in one or more administrations, applications or dosages.

[0020] An "autoimmune disorder" is any condition in which an organism produces antibodies or immune cells which recognize the organism's own molecules, cells or tissues. Non-limiting examples of autoimmune disorders include rheumatoid arthritis,

Sjögren's syndrome, graft versus host disease, myasthenia gravis, and systemic lupus erythematosus.

[0021] An "inflammatory condition" shall mean those conditions that are characterized by a persistent inflammatory response with pathologic sequelae. This state is characterized by infiltration of mononuclear cells, proliferation of fibroblasts and small blood vessels, increased connective tissue, and tissue destruction. Chronic inflammatory diseases include Crohn's disease, psoriasis, and asthma, are also included within the term "inflammatory condition." Autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus can also result in a chronic inflammatory state.

[0022] As used herein, to "treat" includes systemic amelioration of the symptoms associated with the pathology and/or a delay in onset of symptons. Clinical and sub-clinical evidence of "treatment" will vary with the pathology, the individual and the treatment. For example, administration for the treatment of arthritic conditions can result in decreased blood vessel formation in cartilage, specifically joints, resulting in increased mobility and flexibility in these regions. For the treatment of psoriasis, administration will reduce dermatological symptoms such as scabbing, flaking and visible blood vessels under the surface of the skin.

[0023] *In vitro* treatment includes induction of apoptosis, as well as clinical (histological) and sub-clinical (e.g., biochemical and genetic changes associated with a reversal or dimunition of the pathological state.) Clinical and sub-clinical evidence of "treatment" will vary with pathology, the individual or subject, the cell or tissue type and the treatment.

[0024] "An endogenous intracellular enzyme" is one that is expressed by the cell whose regulation or expression can vary. In one aspect, the enzyme selectively activates a compound whose product inhibits proliferation of the cells or kills them. In one aspect, the enzyme is overexpressed in a diseased cell as compared to a normal healthy cell. An example of such is thymidylate synthase (TS).

[0025] A "composition" is intended to mean a combination of active agent and another compound or composition, inert (for example, a solid support, a detectable agent or label) or active, such as an adjuvant.

[0026] A "pharmaceutical composition" is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

[0027] As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin, REMINGTON'S PHARMACEUTICAL SCIENCES, 15th Ed., Mack Publ. Co., Easton, PA (1975).

Methods of Treatment

[0028] Autoimmune diseases and inflammatory conditions are treated by contacting the cells or tissue associated with these pathologies with an effective amount of a compound selected from the group consisting of a 1,5- substituted pyrimidine derivative or analog and furano-pyrimidone derivative or analog.

[0029] When practiced in a subject other than a human patient such as a mouse, the method provides an animal model for use in discovering alternative agents and therapies. In a human patient, the method treats an autoimmune disorder or inflammatory condition. Methods for detecting clinical and sub-clinical evidence of effective therapy are known in the art. In each of these methods, an effective amount of a compound selected from the group consisting of a 1,5- substituted pyrimidine derivative or analog and furano-pyrimidone derivative or analog, is delivered or administered to the subject, e.g., mouse or human patient.

[0030] Numerous compounds of the class defined as a 1,5- substituted pyrimidine derivative or analog and furano-pyrimidone derivative or analog are useful in the invention methods. The 1,5- substituted pyrimidine derivative or analog is substituted at the 5- position with a group that is extractable from pyrimidine by the endogenous, intracellular enzyme, wherein the substituent at the 1- position is selected from the group consisting of substituted sugar, unsubstituted sugar, substituted thio-sugar, unsubstituted thio-sugar, substituted carbocyclic, unsubstituted carbocyclic, substituted acyclic and unsubstituted acyclic. The 1,5- substituted pyrimidine derivative or analog includes, but is not limited to, a 5'-phosphoryl, 5- substituted deoxyuridine derivative or analog or a 5'-phosphoramidate, 5- substituted deoxyuridine derivative or analog. More specifically, the 1,5- substituted pyrimidine derivative or analog includes, but is not limited to, (*E*)-5-(2-bromovinyl)-2'-deoxy-5'-uridyl phenyl L-alanylphosphoramidate. These compounds and methods to prepare them are provided herein.

[0031] In one aspect, the disease is an autoimmune disease, for example, psoriatic arthritis, atherosclerosis, reactive arthritis, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, graft-versus-host disease, osteoarthritis, glomerulonephritis, Type 1 diabetes, muscular dystrophy, or myasthenia gravis. In another aspect, the disease is an inflammatory condition, for example, psoriasis, asthma, ulcerative colitis, inflammatory bowel disease, scleroderma or Crohn's disease.

Co-Administration

[0032] Co-administration of these compounds with other agents may provide unexpected synergistic therapeutic benefit. In the co-administration methods, the compounds are also useful in reducing deleterious side-effects of known therapies and therapeutic agents, as well as yet to be discovered therapies and therapeutic agents. Agents or drugs that neutralize or prevent the production of tumor necrosis factor-α (TNF-α) such as an anti-TNF-α antibody or soluble TNF-α receptor are examples of agents for co-administration with the compounds. Additional examples include, but are not limited to corticosteriods, non-steroidal anti-inflammatory drugs (N-SAIDS), and anti-rheumatic drugs.

[0033] The use of operative combinations is contemplated to provide therapeutic combinations that may lower total dosage of each component than may be required when each individual therapeutic method, compound or drug is used alone. A reduction in adverse effects may also be noted. Thus, the present invention also includes methods involving co-administration of the compounds described herein with one or more additional active agents or methods. Indeed, it is a further aspect of this invention to provide methods for enhancing other therapies and/or pharmaceutical compositions by co-administering a compound of this invention. In co-administration procedures, the agents may be administered concurrently or sequentially. In one embodiment, the compounds described herein are administered prior to the other active agent(s), therapy or therapies. The pharmaceutical formulations and modes of administration may be any of those described herein or known to those of skill in the art.

Use of Compounds for Preparing Medicaments

[0034] The compounds of the present invention are also useful in the preparation of medicaments to treat a variety of autoimmune diseases or inflammatory conditions. The methods and techniques for preparing medicaments of a compound are known in the art.

For the purpose of illustration only, pharmaceutical formulations and routes of delivery are detailed below.

[0035] Thus, one of skill in the art would readily appreciate that any one or more of the compounds described more fully below, including the many specific embodiments, can be used by applying standard pharmaceutical manufacturing procedures to prepare medicaments to treat the many disorders described herein. Such medicaments can be delivered to the subject by using delivery methods known in the pharmaceutical arts.

Pharmaceutical Delivery

[0036] Various delivery systems are known and can be used to administer a compound or an agent of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, receptor-mediated endocytosis and the like. Methods of delivery include but are not limited to, intra-arterial, intramuscular, intravenous, intranasal, and oral routes. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions locally to the area in need of treatment; this may be achieved by, for example and not by way of limitation, local infusion during surgery, by injection, or by means of a catheter. To determine patients that can be beneficially treated, a tissue sample can be removed from the patient and the cells are assayed for sensitivity to the agent.

[0037] Therapeutic amounts can be empirically determined and will vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the compound as well as whether the compound is used alone or in combination with other agents of therapeutic methods. When delivered to an animal, the method is useful to further confirm efficacy of the agent. One example of an animal model is MLR/MpJ-*lpr/lpr* ("MLR-*lpr*") (available from Jackson Laboratories, Bal Harbor, Maine). MLR-*lpr* mice develop systemic autoimmune disease.

[0038] Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

[0039] Suitable dosage formulations and methods of administering the agents can be readily determined by those of skill in the art. For example, the compounds are

administered at about 0.01 mg/kg to about 200 mg/kg, alternatively at about 0.1 mg/kg to about 100 mg/kg, or alternatively at about 0.5 mg/kg to about 50 mg/kg. When the compounds described herein are co-administered with another agent (*e.g.*, as sensitizing agents) or therapy, the effective amount may be less than when the agent is used alone.

[0040] The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may also take the form of suspensions, solutions and emulsions of the active ingredient in aqueous or nonaqueous diluents, syrups, granulates or powders. In addition to an agent of the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compounds of the invention.

[0041] More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including oral, rectal, nasal, topical (including transdermal, aerosol, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It will also be appreciated that the preferred route will vary with the condition and age of the recipient, and the disease being treated.

[0042] Ideally, the agent should be administered to achieve peak concentrations of the active compound at sites of disease. This may be achieved, for example, by the intravenous injection of the agent, optionally in saline, or orally administered, for example, as a tablet, capsule or syrup containing the active ingredient.

[0043] Desirable blood levels of the agent may be maintained by a continuous infusion to provide a therapeutic amount of the active ingredient within disease tissue.

Screening Assays

[0044] This invention also provides a quick and simple screening assay to enable initial identification of novel compounds and combinations useful to treat or ameliorate symptoms of autoimmune and/or chronic inflammatory conditions.

[0045] In one aspect, the assay requires contacting a first sample comprising suitable cells or tissue ("control sample") with an effective amount of a compound selected from the group consisting of a deoxyuridine, a substituted deoxyuridine, a substituted deoxyuridine derivative and analogs thereof and contacting a second sample of the suitable cells or

tissue ("test sample") with the agent to be assayed. In a further aspect, the test agent is contacted with a third sample of cells or tissue comprising normal counterpart cells or tissue to the control and test samples and selecting agents that treat the second sample of cells or tissue but does not adversely effect the third sample. For the purpose of the assays described herein a suitable cell or tissue is one involved in pathogenesis of autoimmune or chronic inflammatory conditions. Examples include, but are not limited to synovial fluid, a chondrocyte or an immune cell, such as a T cell, a macrophage, and an NK cell.

[0046] In a further aspect, the cells are tissue are characterized by the loss of a native tumor suppressor function.

[0047] In yet a further aspect, the assay requires at least two cell types, the first being a suitable control cell. The second cell type is of the same type or tissue as the control cell but differs in that pathogenesis toward disease has begun. In one aspect, pathogenesis is determined enzymatically by noting enhanced or over expression of an endogenous intracellular enzyme that activates the compound into a toxic entity. For example, the compound or agent to be tested can be activated by an endogenous intracellular enzyme that is overexpressed or differentially expressed in a pathological cell as compared to its normal counterpart. An example of such an enzyme includes, but is not limited to thymidylate synthase. Alternatively, a cell genetically modified to differentially express the enzyme or enzymes (containing the appropriate species of enzyme) can be used. Transfection of host cells with polynucleotides encoding the enzyme can be either transient or permanent using procedures well known in the art and described by Chen, L. et al. (1996), Hudziak, R.M. et al. (1988), or Carter, P. et al. (1992), and in the experimental section below. The cells can be procaryotic (bacterial such as *E. coli*) or eucaryotic. The cells can be mammalian or non-mammalian cells, *e.g.*, mouse cells, rat cells, human cells, fungi (*e.g.*, yeast) or parasites (*e.g.*, *Pneumocystis* or *Leishmania*) which cause disease.

[0048] Suitable vectors for insertion of the cDNA are commercially available from Stratagene, La Jolla, CA and other vendors. The amount of expression can be regulated by the number of copies of the expression cassette introduced into the cell or by varying promoter usage. The level of expression of enzyme in each transfected cell line can be monitored by immunoblot and enzyme assay in cell lysates, using monoclonal or polyclonal antibody previously raised against the enzyme for immuno-detection. (Chen, L. et al. (1996)). Enzymatic assays to detect the amount of expressed enzyme also can be

performed as reviewed by Carreras, C.W. and Santi, D.V. (1995), or the method described in the experimental section below.

[0049] In a further aspect, more than one species of enzyme can be used to separately transduce separate host cells, so that the effect of the candidate drug with an enzyme can be simultaneously compared to its effect on another enzyme or a corresponding enzyme from another species.

[0050] The compositions can be directly added to the cell culture media and the target cell or the culture media is then assayed for the amount of label released from the candidate prodrug if the prodrug contains a detectable label. Alternatively, cellular uptake may be enhanced by packaging the prodrug into liposomes using the method described in Lasic, D.D. (1996) or combined with cytofectins as described in Lewis, J.G. et al. (1996).

[0051] The assays are useful to predict whether a subject will be suitably treated by this invention by delivering a compound or composition to a sample containing the cell to be treated and assaying for treatment which will vary with the pathology. In one aspect, the cell or tissue is obtained from the subject or patient by biopsy. Applicants provide kits for determining whether a pathological cell or a patient will be suitably treated by this therapy by providing at least one composition of this invention and instructions for use.

Kits

[0052] Applicants also provide kits for determining whether a pathological cell, tissue or patient will be suitably treated by this therapy. Additionally, kits for performance of the assays are provided. These kits contain at least one composition of this invention and instructions for use.

The Compounds

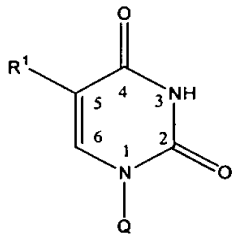
[0053] Therapeutic compounds for use in the methods of this invention are one or more selected from the group consisting a 1,5- substituted pyrimidine derivative or analog and a substituted furano-pyrimidone derivative or analog. In one aspect, the 1,5- substituted pyrimidine derivative or analog is substituted at the 5- position with a group that is extractable from pyrimidine by an endogenous, intracellular enzyme. The substituent at the 1- position is selected from the group consisting of substituted sugar, unsubstituted sugar, substituted thio-sugar, unsubstituted thio-sugar, substituted carbocyclic, unsubstituted carbocyclic, substituted acyclic and unsubstituted acyclic. The 1,5-

substituted pyrimidine derivative or analog includes, but is not limited to, a 5'-phosphoryl, 5- substituted deoxyuridine derivative or analog or a 5'- phosphoramidate, 5- substituted deoxyuridine derivative or analog. More specifically, the 1,5- substituted pyrimidine derivative or analog includes, but is not limited to, (*E*)-5-(2-bromovinyl)-2'-deoxy-5'-uridyl phenyl L-alanylphosphoramidate.

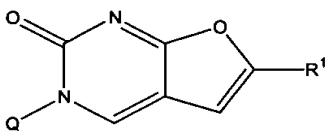
[0054] In a further embodiment, the compounds are not chemically related to pyrimidines or folates, and can be synthesized based upon known parameters of rational drug design. See Dunn, W.J. et al. (1996).

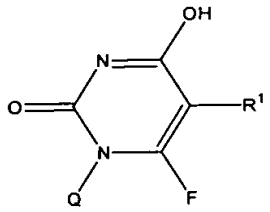
[0055] Compounds useful in the methods of this invention can be described as the L and D isomers of compounds having one of the following structures:

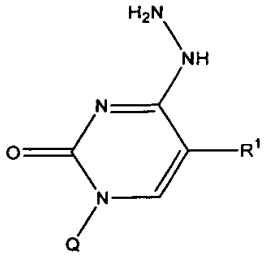
Formula A



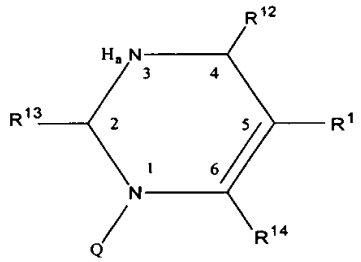
Formulae B







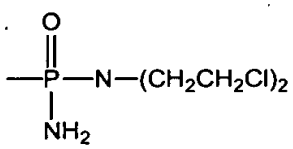
or Formula C



[0056] or tautomers thereof, wherein in Formula C, R^{12} or R^{13} may be the same or different and are selected from the group consisting of oxo, OH or $NHNH_2$, wherein a is 0 or 1, providing that if a is 0 and R^{13} is oxo, then a double bond exits between position 3 and 4 and R^{12} is $NHNH_2$; further providing that if a is 0 and R^{12} is oxo, then a double bond exists between position 2 and 3 and R^{13} is $NHNH_2$; further providing that if a is 1, then R^{12} and R^{13} are both oxo.

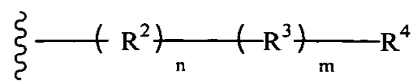
[0057] While not wishing to be bound by any theory, in one aspect of the above formulae (A, B and C), R^1 (at the 5-position) is or contains a leaving group which is a chemical entity that has a molecular dimension and electrophilicity compatible with extraction from the pyrimidine ring by an endogenous, intracellular enzyme (*e.g.*, thymidylate synthase). An embodiment for the substituent in the R^1 position is one that could undergo an allylic interchange.

[0058] Annother example is an alkenyl group of the formula, *i.e.*, $(-CH=CH)_n-R^4$, wherein n is 0 or an integer from 1 to 10, and R^4 is a halogen such as I or Br, CN or mercury, or alternatively, R^1 is or contains a group selected from hydrogen, alkyl, alkene, alkyne, hydroxy, -O-alkyl,-O-aryl, O-heteroaryl, -S-alkyl, -S-aryl, a cyanide, cyanate, thiocyanate halovinyl group, halomercuric group, -S-heteroaryl, $-NH_2$, -NH-alkyl, $-N(alkyl)_2$, -NHCHO, -NHOH, -NHO-alkyl, NH_2CONHO-, and $NHNH_2$. For example, when n is 0 or an integer from 1 to 10, R^4 is $-CH_2$-O-A, wherein A is a phosphoramide derivative, or a compound of the formula:



[0059] Alternatively, in the above formulae (A, B or C), R^1 can be a moiety of the formula:

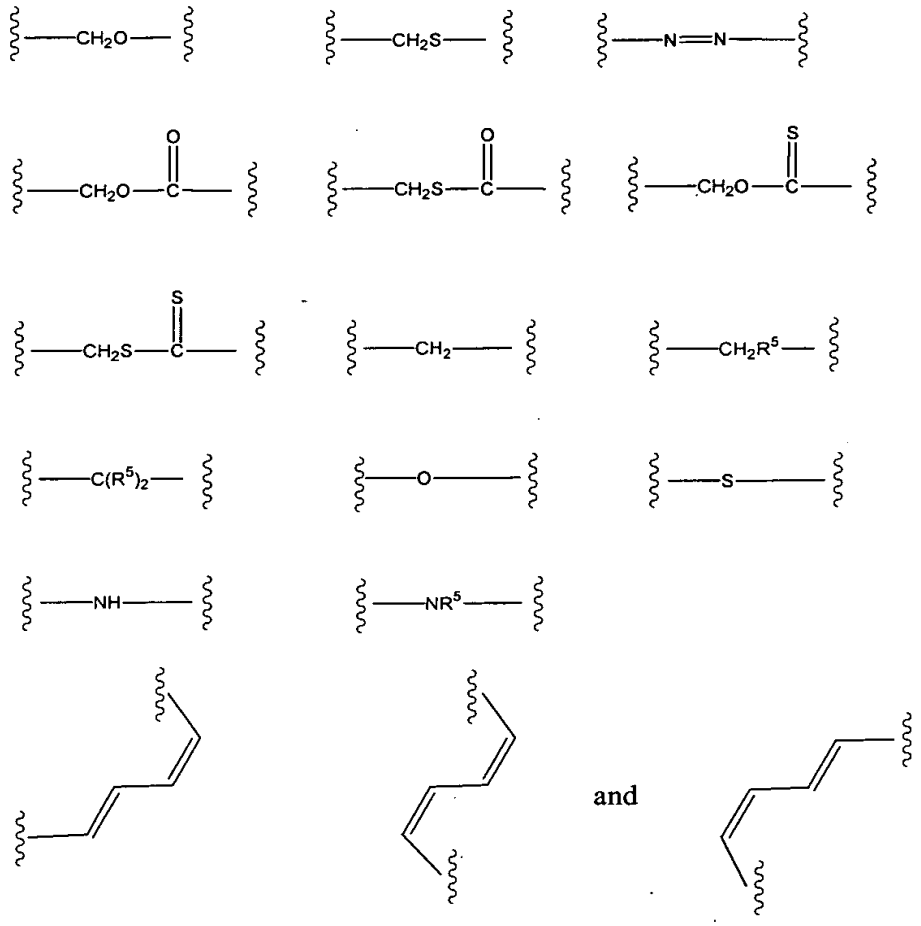
Formula D



[0060] wherein, R^4 is a toxophore.

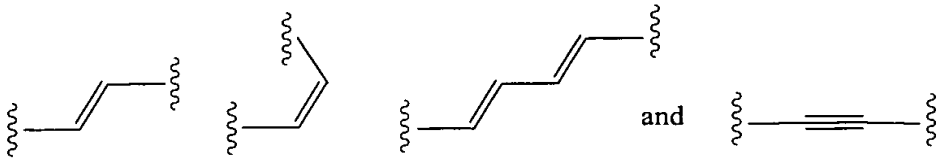
[0061] In one aspect of Formula D, R^2 is or contains a divalent electron conduit moiety. In one embodiment, R^2 is or contains a mono- or polyunsaturated electron conduit acting to conduct electrons away from the pyrimidine ring and toward R^4. In one embodiment, R^2 is selected from the group consisting of an unsaturated hydrocarbyl group, an aromatic hydrocarbyl group comprising one or more unsaturated hydrocarbyl groups, and a heteroaromatic group comprising one or more unsaturated hydrocarbyl groups.

[0062] In a yet further aspect, m is 0 and R^2 is selected from the group consisting of:

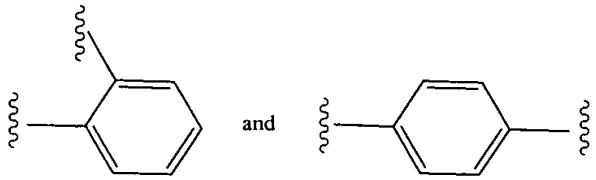


[0063] wherein R^5 is independently the same or different and is selected from the group consisting of a linear or branched alkyl group having from 1 to 10 carbon atoms, a cycloalkyl group having from 3 to 10 carbon atoms, CN and a halogen.

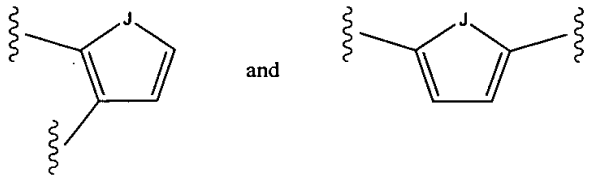
[0064] In one embodiment of Formula D, R^2 is an unsaturated hydrocarbyl group having a structure selected from the group consisting of:



[0065] In another embodiment of Formula D, R^2 is an aromatic hydrocarbyl group having a structure selected from the group consisting of:



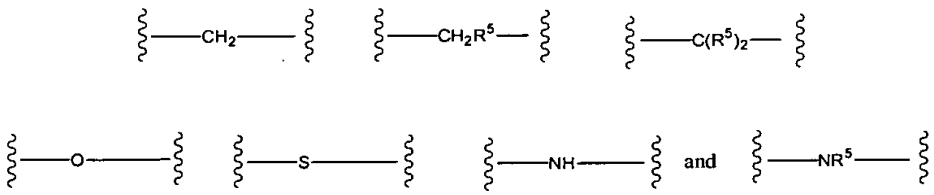
[0066] In yet another embodiment of Formula D, R^2 is a heteroaromatic group having a structure selected from the group consisting of:



[0067] wherein J is a heteroatom, such as -O-, -S-, or -Se-, or a heteroatom group, such as -NH- or $-NR^{ALK}-$, where R^{ALK} is a linear or branched alkyl having 1 to 10 carbon atoms or a cycloalkyl group having 3 to 10 carbon atoms.

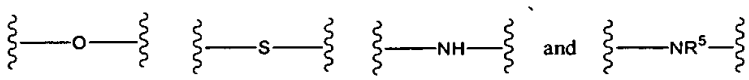
[0068] In an alternative embodiment of Formula D, R^3 is a divalent spacer moiety, also referred to as a spacer unit. Divalent spacers include, but are not limited to, a moiety having a structure:

Formulae E

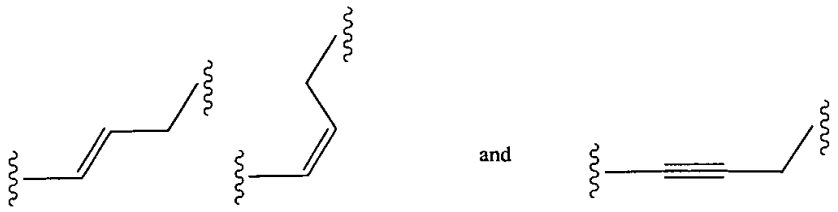


[0069] wherein R^5 is the same or different and is independently a linear or branched alkyl group having from 1 to 10 carbon atoms, or a cycloalkyl group having from 3 to 10 carbon atoms.

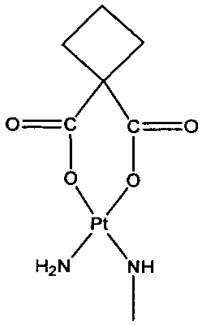
[0070] In an alternative aspect of Formula D, R^3 is a divalent spacer moiety having a structure selected from the group consisting of:



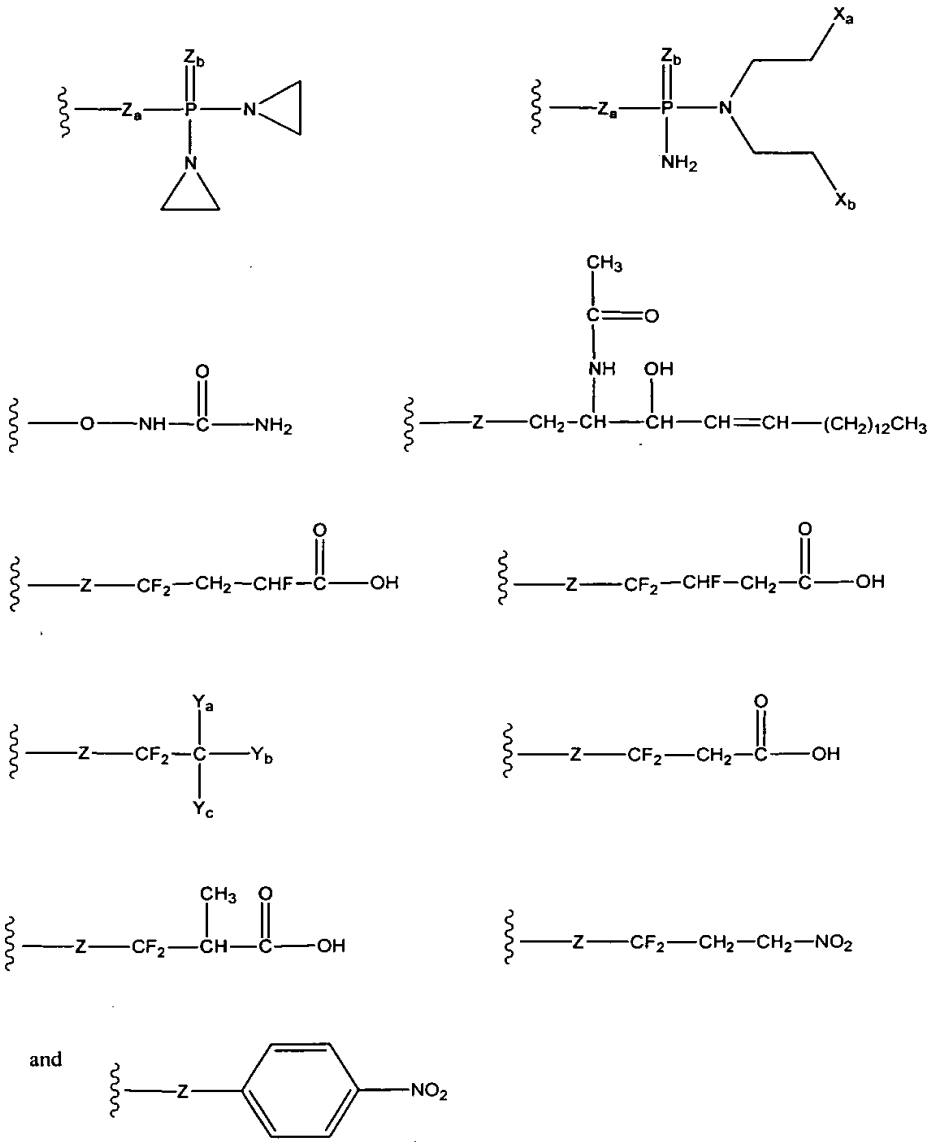
[0071] In yet another aspect of Formula D, R^2 and R^3, taken together form a structure selected from the group consisting of:



[0072] In one embodiment, R^4 (R^4 in Formula D or R^1 in Formulae A, B or C) is or contains a leaving group that is activated or released by an intracellular enzyme. In one embodiment, R^4 is or contains a group having a structure selected from the group consisting of F, Cl, Br, I, CN, SO_3H, CO_2H, $CO_2CH_2CH_3$, CO_2CH_3, $SI(CH_3)_3$, CHO, NO_2, CF_3, CCl_3, $CH=C(R^{15})_2$ and a derivative of cisplatin, such as:



[0073] or a substituent selected from the structures:

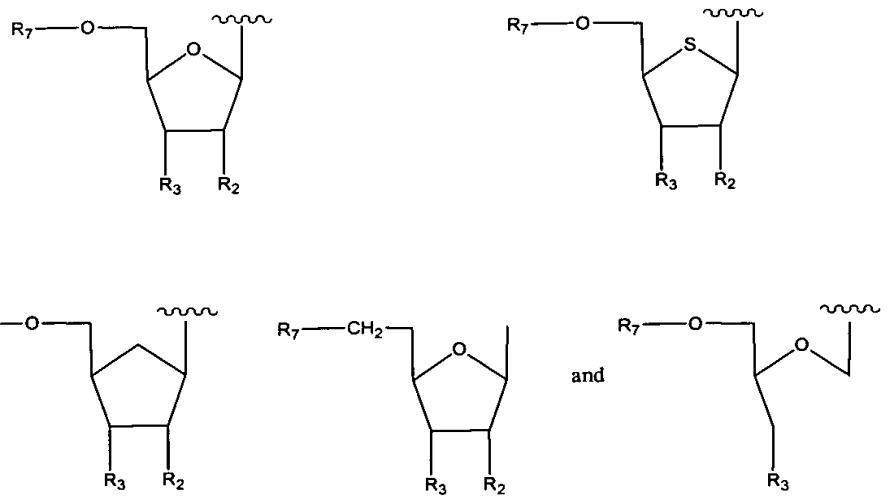


[0074] wherein X_a and X_b are independently the same or different and are selected from the group consisting of Cl, Br, I, and a potent leaving group and wherein Y_a, Y_b or Y_c are independently the same or different and are hydrogen or F and wherein Z, Z_a and Z_b are independently the same or different and are selected from the group consisting of O and S; and with respect to Formula C, R^{14} is hydrogen or F, providing if R^{14} is F, then a is 1 and R^{12} and R^{13} are both oxo.

[0075] In a further aspect, Q is a sugar group, a thio-sugar group, a carbocyclic group or an acyclic carbon group as well as 5'-phosphoryl or phosphoramidate derivatives thereof. Examples of sugar groups include, but are not limited to, monosaccharide cyclic sugar groups such as those derived from oxetanes (4-membered ring sugars), furanoses (5-membered ring sugars), and pyranoses (6-membered ring sugars). Examples of furanoses include threo-furanosyl (from threose, a four-carbon sugar); erythro-furanosyl (from erythrose, a four-carbon sugar); ribo-furanosyl (from ribose, a five-carbon sugar); ara-furanosyl (also often referred to as arabino-furanosyl; from arabinose, a five-carbon sugar); xylo-furanosyl (from xylose, a five-carbon sugar); and lyxo-furanosyl (from lyxose, a five-carbon sugar). Examples of sugar group derivatives include "deoxy", "keto", and "dehydro" derivatives as well as substituted derivatives. Examples of thio sugar groups include the sulfur analogs of the above sugar groups, in which the ring oxygen has been replaced with a sulfur atom. Similar substitutions can be made to the acyclic carbon group. Examples of carbocyclic groups include C_4 carbocyclic groups, C_5 carbocyclic groups, and C_6 carbocyclic groups which may further have one or more substituents, such as -OH groups.

[0076] In one embodiment, Q is selected from the group consisting of:

Formulae F

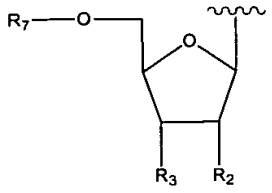


[0077] In the above Formula F, R_2 and R_3 are independently the same or different and are selected from the group consisting of Br, Cl, F, I, H, OH, $OC(=O)CH_3$, -O-and -O-Rg,

[0078] wherein Rg is a hydroxyl protecting group other than acetyl. R_7 is attached to Q at the 5' position of Q and is selected from the group consisting of a hydrogen, a hydroxyl, a phosphate group, a phosphodiester group or a phosphoramidate group. R_7 is selected from the group consisting of a hydrogen, a masked phosphate, a phosphoramidate, and derivatives thereof, and wherein R_2 and R_3 are the same or different and are independently hydrogen , -OH -OC(=O)CH_3, or -O-Rg wherein Rg is a hydroxyl protecting group other than acetyl. Any of the members of Formulae F may be in any enantiomeric, diasteriomeric, or stereoisomeric form, including D-form, L-form, α-anomeric form, and β-anomeric form.

[0079] In a specific embodiment, Q has the formula:

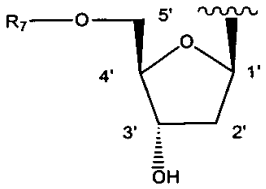
Formula G



[0080] wherein R_2 and R_3 are independently the same or different and are independently H, -OH, -OC(=O)CH_3, or –O-Rg, wherein Rg is a hydroxyl protecting group other than acetyl.

[0081] In a further specific embodiment, Q has the following structure:

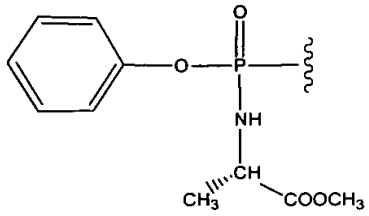
Formula H



[0082] In each of Formulae F, G, or H, R_7 is selected from the group consisting of hydrogen, a masked phosphate or a phosphoramidate and derivatives thereof, and wherein R_2 and R_3 are the same or different and are independently hydrogen or -OH. Alternatively, R_7 is a phosphoramidate group derived from an amino acid, including, for

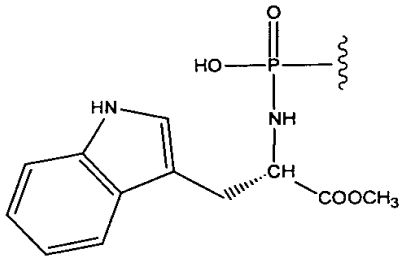
example, the twenty naturally occurring amino acids, *e.g.*, alanine and tryptophane. Examples of such include, but are not limited to:

Formula I

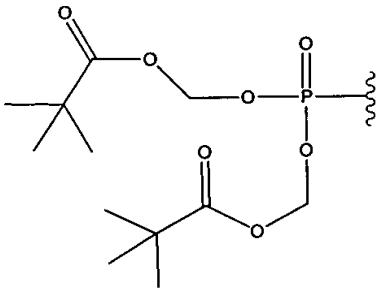


[0083] Formula I and its method for preparation, are described in McGuigan, C. et al. (1993), and McGuigan, C. et al. (1996). Additional examples of 5' substituents are:

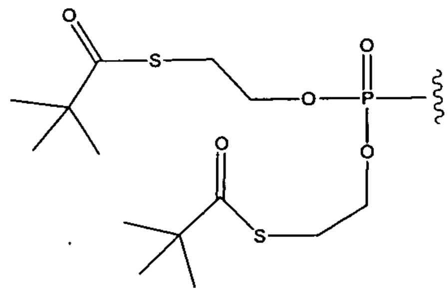
Formula J



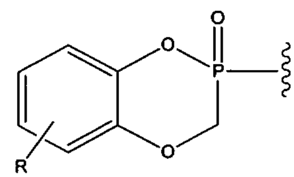
Formula K



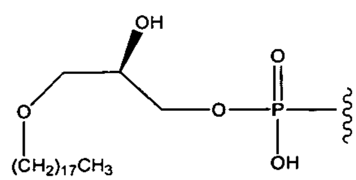
Formula L



Formula M



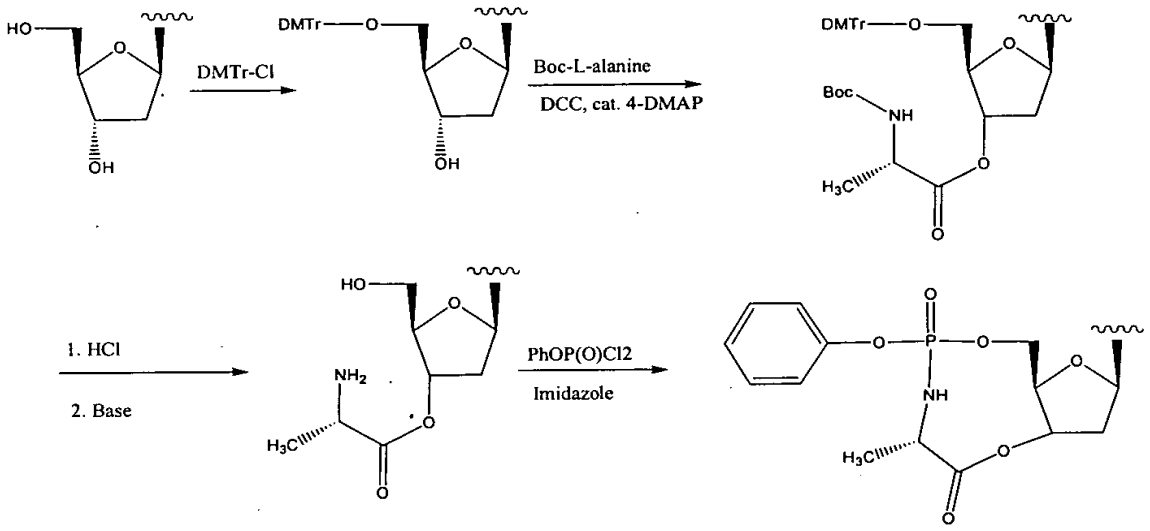
and Formula N



[0084] The group identified herein as Formula J, and methods for its preparation, are described in Abraham et al. (1996). Formula K and its method for preparation are described in Freed et al. (1989); Sastry et al. (1992); Farquhar, J. et al. (1994), and Farquhar, J. et al. (1995). Formula L and its method for preparation are described in Valette et al. (1996); and Benzaria et al. (1996). Formula M and its method of preparation are described in Meier et al. (1997); Meier et al. (1997); and Meier et al. (1997). Formula N and its method for preparation, are described in Hostetler et al. (1997); and Hostetler et al., published International Patent Application No. WO 96/40088 (1996).

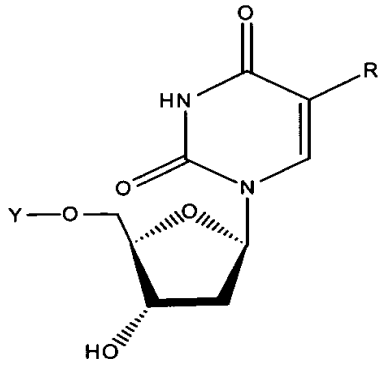
[0085] In one embodiment, the R_7 forms a cyclic group within Q. One such embodiment, and a method for its preparation, is shown below (where DMTr is 4,4'-dimethoxytrityl,

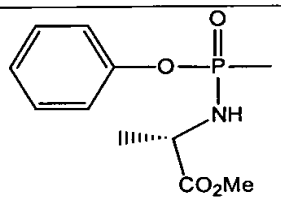
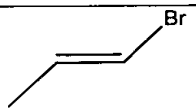
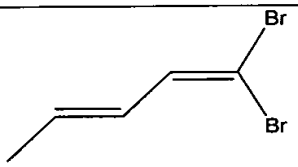
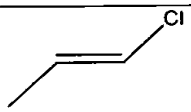
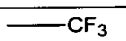
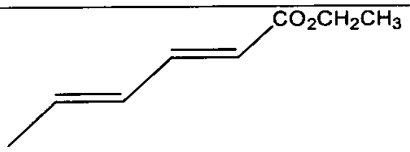
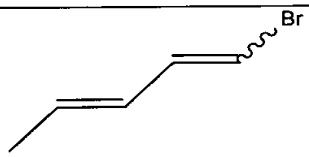
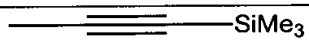
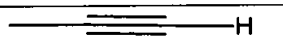
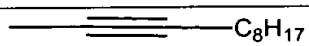
Boc is t-butyloxycarbonyl, DCC is 1,3-dicyclohexylcarbodiimide, and 4-DMAP is 4-dimethylaminopyridine):



[0086] In one embodiment, the compound may be in any enantiomeric, diasteriomeric, or stereoisomeric form, including, D-form, L-form, α-anomeric form, and β-anomeric forms. In an alternative embodiment, the compound may be in a salt form, or in a protected or prodrug form, or a combination thereof, for example, as a salt, an ether, or an ester.

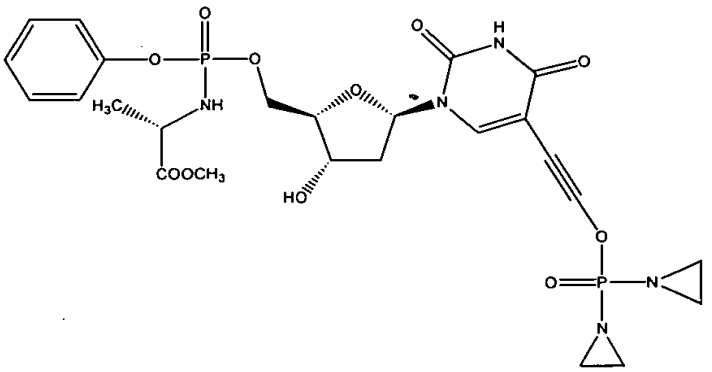
[0087] Specific compounds having the L or D structures are shown in Table I, below. Compounds are identified by structure and a numerical designation.



R	Y= 	Y=H
	NB 1011	NB 1015 (BVdU)
	NB 1012	--
	NB 1013	NB 1020
	NB 1014	NB 1027
	NB 1016	NB 1021
	NB 1017	NB 1024
	NB 1018	NB 1022
	NB 1019	NB 1023
	--	--
$-C_8H_{17}$	--	--

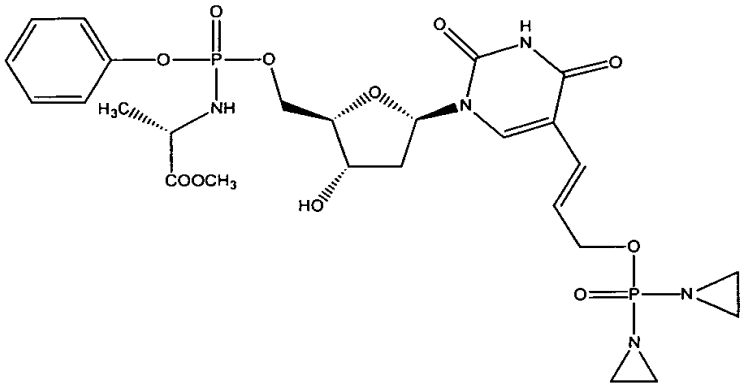
[0088] The structures of specific examples of compounds useful in the methods of this invention are provided below.

[0089] For example, a compound having the structure:



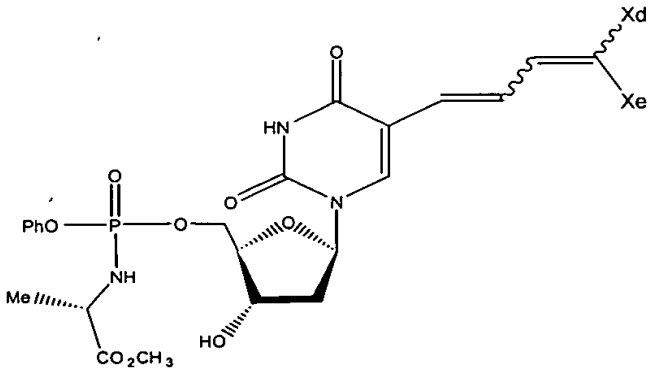
or the nucleoside analog thereof.

[0090] A compound having the structure:



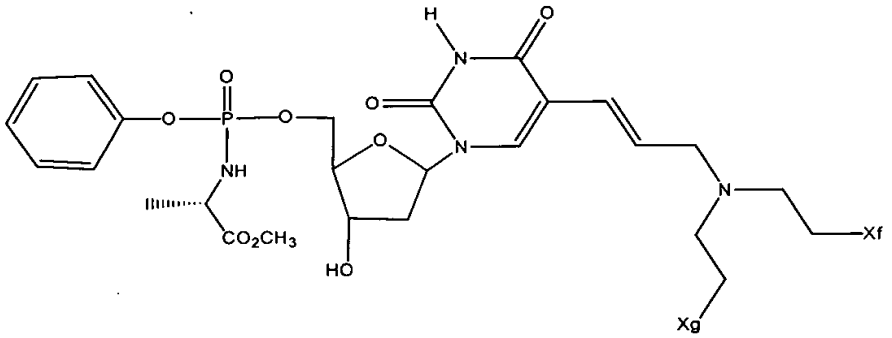
or the nucleoside analog thereof.

[0091] A compound having the structure:



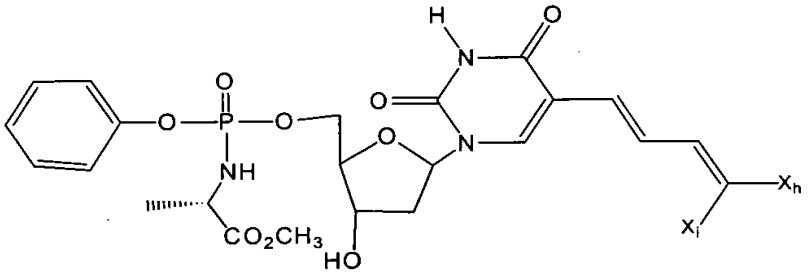
[0092] wherein X_d and X_e are independently the same or different and are selected from the group consisting of Cl, Br, I, and CN or the nucleoside analogs thereof. In a more preferred aspect, X_d is Cl or Br and X_e is hydrogen.

[0093] A compound having the structure:



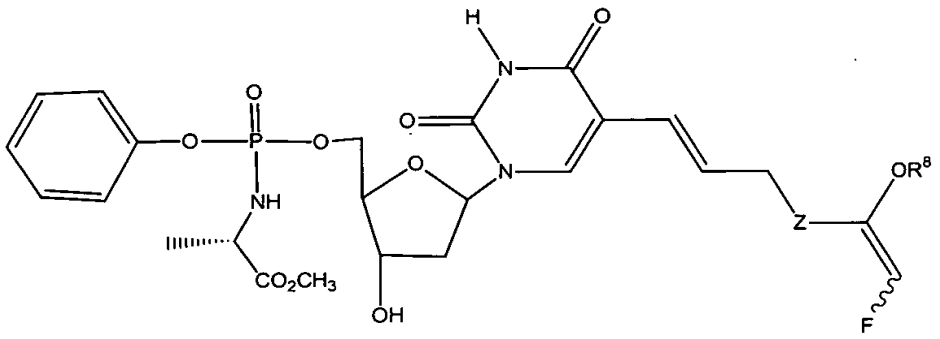
wherein X_f and X_g are independently the same or different and are selected from the group consisting of Cl, Br, I, and CN, or the nucleoside analogs thereof. In a preferred embodiment, X_f and X_g are the same and are each is Cl or Br.

[0094] A compound having the structure of the formula:



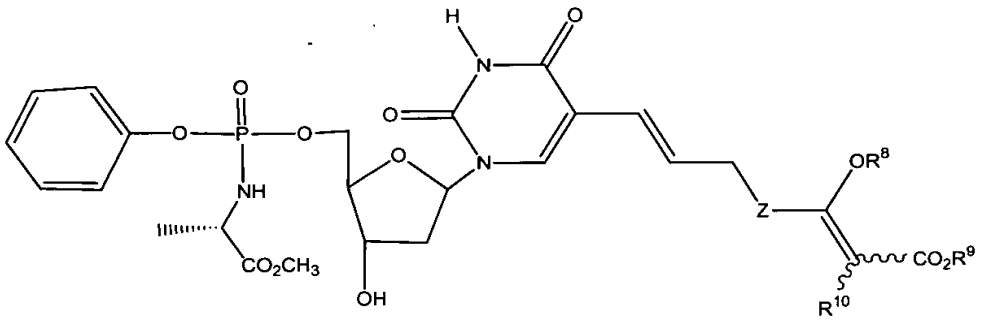
[0095] wherein X_h and X_i are independently the same or different and are selected from the group consisting of Cl, Br, I, and CN, or the nucleoside analogs thereof. In a preferred embodiment, X_h and X_i are independently the same or different and are Cl or Br and in a more preferred embodiment, X_h and X_i are both Br.

[0096] A compound having the structure:



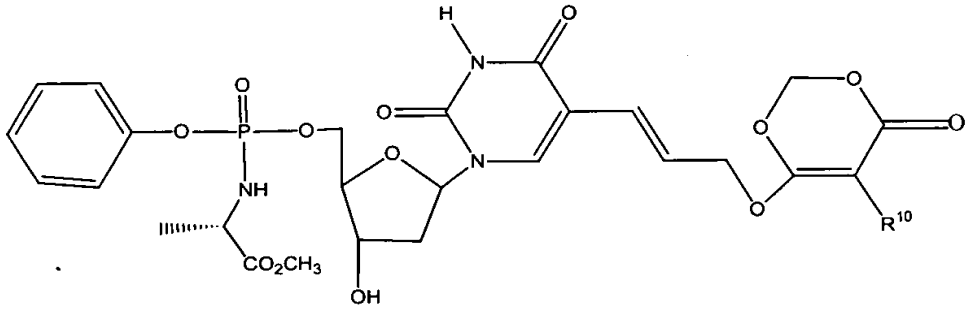
wherein R^8 is a lower straight or branched chain alkyl, or the nucleoside analogs thereof.

[0097] A compound having the structure:



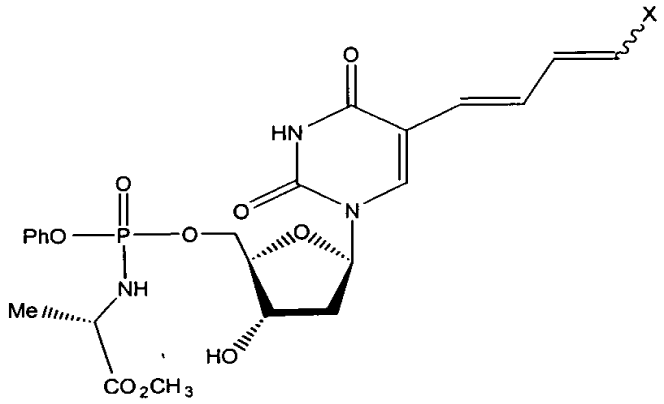
[0098] wherein R^8 and R^9 are lower straight or branched chain alkyls and R^{10} is hydrogen or CH_3, or the nucleoside analogs thereof.

[0099] A compound having the structure:



wherein R^{10} is hydrogen or CH_3,or the nucleoside analogs thereof.

[00100] A compound having the structure:



[00101] wherein X is selected from the group consisting of CO_2Et, Cl, and Br; or the nucleoside analogs thereof.

[00102] In a separate embodiment, the above structures are further modified to possess thiophosphodiaziridine instead of phosphodiaziridine groups, using the methods described below.

[00103] The compounds can be combined with a carrier, such as a pharmaceutically acceptable carrier, for use *in vitro* and *in vivo*. In one embodiment, the compound is in a salt form, or in a protected or prodrug form, or a combination thereof, for example, as a salt, an ether, or an ester.

[00104] "Pharmaceutically acceptable salt, prodrug or derivative" as used herein, relates to any pharmaceutically acceptable salt, ester, ether, salt of an ester, solvate, such as ethanolate, or other derivative of a compound of the present invention which, upon administration to a recipient, is capable of providing (directly or indirectly in the case of a prodrug) a compound of this invention or an active metabolite or residue thereof. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (*e.g.*, by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (*e.g.*, the brain or lymphatic system).

[00105] Salts of the prodrugs of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicyclic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic,

benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, can be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Examples of bases include alkali metal (*e.g.*, sodium) hydroxides, alkaline earth metal (*e.g.*, magnesium) hydroxides, ammonia, and compounds of formula NW_4^+, wherein W is C_{1-4} alkyl.

[00106] Examples of salts include: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylproprionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4} alkyl group).

[00107] For therapeutic use, salts of the compounds of the present invention will be pharmaceutically acceptable. However, salts of acids and bases which are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

[00108] Esters of the prodrugs or compounds identified by the method of this invention include carboxylic acid esters (*i.e.*, -O-C(=O)R) obtained by esterification of the 2'-, 3'- and/or 5'-hydroxy groups, in which R is selected from (1) straight or branched chain alkyl (for example, n-propyl, t-butyl, or n-butyl), alkoxyalkyl (for example, methoxymethyl), aralkyl (for example, benzyl), aryloxyalkyl (for example, phenoxymethyl), aryl (for example, phenyl optionally substituted by, for example, halogen, C_{1-4}alkyl, or C_{1-4}alkoxy or amino); (2) sulfonate esters, such as alkylsulfonyl (for example, methanesulfonyl) or aralkylsulfonyl; (3) amino acid esters (for example, L-valyl or L-isoleucyl); (4) phosphonate esters and (5) mono-, di- or triphosphate esters. The phosphate esters may be further esterified by, for example, a C_{1-20} alcohol or reactive derivative thereof, or by a 2,3-di-(C_{6-24})acyl glycerol. In such esters, unless otherwise specified, any alkyl moiety present advantageously contains from 1 to 18 carbon atoms, particularly from 1 to 6 carbon atoms, more particularly from 1 to 4 carbon atoms. Any cycloalkyl moiety present

in such esters advantageously contains from 3 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group. Examples of lyxo-furanosyl prodrug derivatives of the present invention include, for example, those with chemically protected hydroxyl groups (*e.g.*, with O-acetyl groups), such as 2'-O-acetyl-lyxo-furanosyl; 3'-O-acetyl-lyxo-furanosyl; 5'-O-acetyl-lyxo-furanosyl; 2',3'-di-O-acetyl-lyxo-furanosyl and 2',3',5'-tri-O-acetyl-lyxo-furanosyl.

[00109] Ethers of the compounds of the present invention include methyl, ethyl, propyl, butyl, isobutyl, and sec-butyl ethers.

Formulations for *In Vivo* Administration

[00110] While it is possible for the composition ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation comprising at least one active ingredient, as defined above, together with one or more pharmaceutically acceptable carriers therefore and optionally other therapeutic agents. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

[00111] Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented a bolus, electuary or paste.

[00112] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (*e.g.*, povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (*e.g.*, sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) and/or surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release

profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

[00113] Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[00114] Pharmaceutical compositions for topical administration according to the present invention may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol or oil. Alternatively, a formulation may comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active ingredients and optionally one or more excipients or diluents.

[00115] For diseases of the eye or other external tissues, *e.g.*, mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient in an amount of, for example, about 0.075 to about 20% w/w, preferably about 0.2 to about 25% w/w and most preferably about 0.5 to about 10% w/w. When formulated in an ointment, the composition may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the ingredients may be formulated in a cream with an oil-in-water cream base.

[00116] If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, *i.e.*, an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound that enhances absorption or penetration of the ingredients through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

[00117] The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While this phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier that acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil

and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

[00118] Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

[00119] The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

[00120] Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the ingredients. The ingredients are preferably present in such formulation in a concentration of about 0.5 to about 20%, advantageously about 0.5 to about 10%, particularly about 1.5% w/w.

[00121] Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

[00122] Formulations suitable for vaginal administration may be presented as suppositories, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the ingredients, such carriers as are known in the art to be appropriate.

[00123] Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered in the manner in which snuff is taken, *i.e.*, by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer, include aqueous or oily solutions of the ingredients.

[00124] Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[00125] It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable of oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

[00126] Compositions of the formula of the present invention may also be presented for the use in the form of veterinary formulations, which may be prepared by methods that are conventional in the art.

[00127] The following examples are intended to illustrate, but not limit, the invention.

MATERIALS AND METHODS

Synthesis of Nucleoside Compounds

[00128] Synthesis of the above noted 5-substituted pyrimidine derivatives can be accomplished by methods known in the art, for example as described in Applicant's patent literature, PCT/US98/16607 and PCT/US99/01332.

[00129] One method requires treatment of 5-chloromercuri-2'-deoxyuridine with haloalkyl compounds, haloacetates or haloalkenes in the presence of Li_2PdCl_4 to form, through an organopalladium intermediate, the 5-alkyl, 5-acetyl or 5-alkene derivative, respectively (Wataya, Y. et al. (1979) and Bergstrom, D. E. et al. (1984)). Another example of C5-modification of pyrimidine nucleosides and nucleotides is the formation of C5-*trans*-styryl derivatives by treatment of unprotected nucleotide with mercuric acetate

followed by addition of styrene or ring-substituted styrenes in the presence of Li_2PdCl_4 (Bigge, et al. (1980)).

[00130] Pyrimidine deoxyribonucleoside triphosphates can be derivatized with mercury at the 5 position of the pyrimidine ring by treatment with mercuric acetate in acetate buffer at 50° for 3 hours (Dale, et al. (1973)). Such treatment also would be expected to be effective for modification of monophosphates. Alternatively, a modified triphosphate can be converted enzymatically to a modified monophosphate, for example, by controlled treatment with alkaline phosphatase followed by purification of monophosphate. Other moieties, organic or nonorganic, with molecular properties similar to mercury but with preferred pharmacological properties could be substituted. For general methods for synthesis of substituted pyrimidines *see*, for example, U.S. Patent Nos. 4,247,544, 4,267,171, and 4,948,882 and Bergstrom, D. E. et al. (1981). The above methods would also be applicable to the synthesis of derivatives of 5-substituted pyrimidine nucleosides and nucleotides containing sugars other than ribose or 2'-deoxyribose, for example 2'-3'-dideoxyribose, arabinose, furanose, lyxose, pentose, hexose, heptose, and pyranose. An example of a 5-position substituent is the halovinyl group, *e.g.* (*E*)-5-(2-bromovinyl)-2'-deoxyuridylate (Barr, P. J. et al. (1983)).

[00131] Alternatively, 5-bromodeoxyuridine, 5-iododeoxyuridine, and their monophosphate derivatives are available commercially from Glen Research, Sterling, VA (USA), Sigma-Aldrich Corporation, St. Louis, MO (USA), Moravek Biochemicals, Inc., Brea, CA (USA), ICN, Costa Mesa, CA (USA) and New England Nuclear, Boston, MA (USA). Commercially-available 5-bromodeoxyuridine and 5-iododeoxyuridine can be converted to their monophosphates either chemically or enzymatically, through the action of a kinase enzyme using commercial available reagents from Glen Research, Sterling, VA (USA) and ICN, Costa Mesa, CA (USA). These halogen derivatives could be combined with other substituents to create novel and more potent antimetabolites.

[00132] In one aspect, the structures at the 5-position of the 1,5- substituted pyrimidine derivatives or analogs in Formulae A, B and C are referred to as the tethers because they connect a proposed leaving group (toxophore) to the heterocycle.

[00133] In one aspect, the tether also contains a spacer between the toxin and the pyrimidine ring can be unsaturated, e.g., vinyl, allyl, and propargyl units are simple, small, and readily accessible synthetically. The vinyl and allyl units have the advantage that they can be prepared in either of two non-interconvertible geometric isomeric forms.

Alternatively, synthesis based on the structure of BVdU monophosphate and features a proposed leaving group/toxin directly attached to the terminus of a (poly)vinyl substituent at C5 of the pyrimidine ring. This is the vinyl tether approach. A yet further approach is based on the structure of TFPe-dUMP and is similar to the vinyl tether approach but has a methylene unit separating the proposed leaving group/toxin and the unsaturated unit and thus contains an allyl or propargyl unit. This is the allyl tether approach.

[00134] 5-Alkylidenated 5,6-dihydrouracils similar in structure to the intermediate common to both the vinyl and allyl tether approach mechanisms have been synthesized recently (Anglada, J. M. et al. 1996). A C5 methylene intermediate produced by the enzyme thymidylate synthase TS was demonstrated by trapping studies (Barrett, J. E. et al. (1998)).

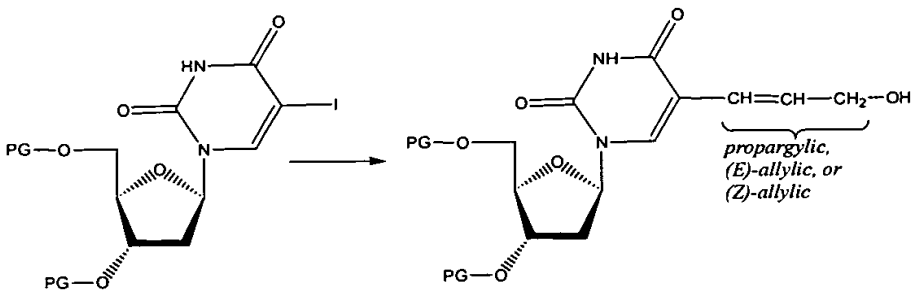
[00135] The compounds of Formula B are defined by the structure of the uracil base, or modified uracil base present. These classes are compounds where: 1) the base is a furano-pyrimidinone derivative of uracil; 2) the base is 6-fluoro uracil; 3) the base is 4-hydrazone substituted uracil derivative; and 4) the base is uracil. In one aspect, the uracil or modified uracil derived base is used to synthesize compounds substituted with toxic leaving groups at the 5 position, attached by an electron conduit tether at this 5 position, and including an appropriate spacer moiety between the electron conduit and the toxic leaving group. The compounds can be unphosphorylated, 5' monophosphate, 5' phosphodiester, or 5' protected ("masked") deoxyuridines or comparable derivatives of alternative carbohydrate moieties, as described below. Protected 5-substituted deoxyuridine monophosphate derivatives are those in which the phosphate moiety has been blocked through the attachment of suitable chemical protecting groups. In another embodiment, 5-substituted uracil or uridine derivatives are administered to cells containing nucleoside kinase activity, wherein the 5-substituted uracil/uridine derivative is converted to a 5-substituted uridine monophosphate derivative. Uridine derivatives may also be modified to increase their solubility, cell penetration, and/or ability to cross the blood-brain barrier.

Synthesis of Compounds with Propargyl Tethers

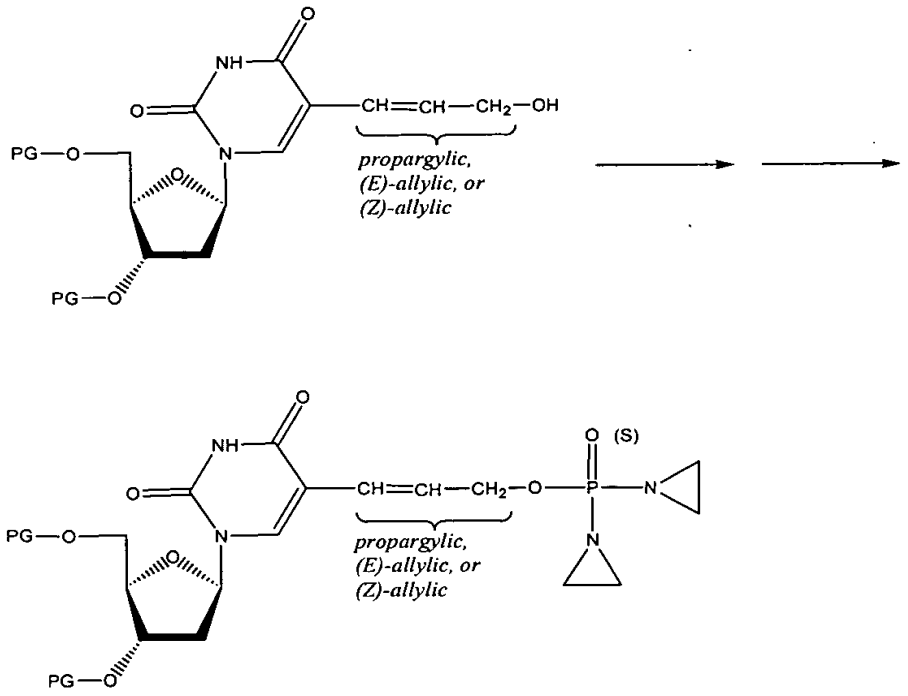
[00136] The synthesis of propargylic and allylic alcohol-equipped 2'-deoxyuridines are reported in the literature. For example, Barr, P. J. and Robins, M. J. (1981) and Balzarini, J. et al. (1985).

[00137] Both 5-mercuri- (Ruth, J. L. et al. (1978)) and 5-iodouridines (Robins, M. J. et al. (1981)) readily condense with alkenes and alkynes in the presence of a palladium catalyst to afford C5 tether-equipped uridines. The latter route is the more often employed (Robins, M. J. et al. (1982) and Asakura, J. et al. (1988) and (1990)). High-yielding condensations of protected 5-iodo-2'-deoxyuridines with t-butyidimethylsilyl propargyl ether (Graham, D. et al. (1998); De Clercq, E. et al. (1983), methyl propargyl ether (Tolstikov, V. V. et al. (1997)) and even propargyl alcohol itself (Chaudhuri, N. C. et al. (1995) and Goodwin, J. T. et al. (1993)) have been achieved. The 3-hydroxy-l-propynyl substituent introduced by the latter reaction can also be accessed by DIBAL-H reduction of a methacrylate group (Cho, Y. M. et al. (1994)), itself arising from the same Heck reaction used in the synthesis of BVdU. These palladium-catalyzed reactions can be used to condense very long and elaborately-functionalized propargyl-based tethers to 5-iodo-2'-deoxyuridines. (Livak, K. J. et al. (1992) and Hobbs, F. W. Jr. (1989)). (*Z*)-Allyl-based tethers are generated by the partial hydrogenation of a propargylic precursor over Undiar catalyst (Robins, M. J. et al. (1983)) whereas the (*E*)-allyl-based ones are best prepared by Heck coupling of an (*E*)-tributylstannylated ethylene (Crisp, G. T. (1989)).

[00138] Closely following the literature procedures, a t-butyldimethylsilyl propargyl ether-equipped 3', 5'-di-O-protected 2'-deoxyuridine (Graham, D. et al. (1998), and De Clercq, E. et al. (1983)) can be prepared and a portion of it, converted to the corresponding (*Z*)-allyl ether, (Robins, M. J. et al. (1983)) is reduced. Because the TBAF-mediated removal of a TBDMS group generates an oxyanion that can be functionalized *in situ*, these TBDMS-protected propargyl- and (*Z*)-allytic-tethered nucleosides can serve as convenient precursors to some of the toxophore-equipped targets. For the (*E*)-allyl alcohol equipped nucleoside, the known O-tetrahydropyranyl ether derivative is prepared by the literature Heck coupling of an (*E*)-tributylstannylated ethylene (Crisp, G. T. (1989)).

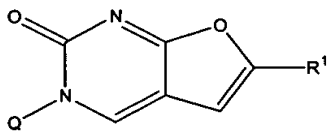


[00139] Using a two step literature protocol (Phelps, M. E. et al. (1980) and Hsiao, L. Y. et al. (1981)), the propargylic and (*E*) and (*Z*)-allylic alcohols are converted to their corresponding bis-aziridinyl phosphoramidates or thiophosphoramidates.



Synthesis of Furano-Pyrimidinones

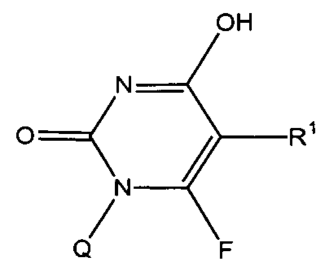
[00140] Synthesis of furano-pyrimidinones begins with synthesis of a C5 propargylic - alcohol-equipped 2'-deoxyuridine. Furano-pyrimidinone compounds are then be formed from the O-tetrahydropyranyl ether derivative described above. Synthesis proceeds by reaction of the second carbon of the propargyl bond with the oxygen attached to the C4 position of the pyrimidine ring to yield a fluorescent furano-pyrimidinone which can be readily separated from the reaction mix. Such compounds provide an additional basis for synthesis of compounds through various combinations of specific electron conduits, spacers and toxic leaving groups.



[00141] Furo[2,3-d]pyrimidinone nucleosides (represented by the above generic structure) were prepared by condensing 2',3'-di-O-p-toluoyl or 2',3'-di-O-acetyl-5-iodo-2'-deoxyuridine with 1-(tetrahydropyranyloxy)-2-propyne (Jones, R. G. and Mann, M. J. (1953)) under conditions known to promote the formation of these fluorescent compounds (Robins, M. J. et al.(1983)). Base-catalyzed removal of the carbohydrate protecting groups gave the 6-(tetrahydropyran-2-yloxymethyl)-substituted bicyclic nucleoside which was either subjected to standard acidic THP group hydrolysis (TFA in CH_2Cl_2) or was regioselectively 5'-phosphoramidated by the same procedure used to prepare BVdU-PA and 5FUdR-PA. After the phosphoramidation, the THP group can be removed by acidic hydrolysis.

Compounds Based on Furano-Pyrimidinones

[00142] Examples of synthesis of compounds having a structure of the class shown are as follows.



[00143] Proposed toxic R^4 leaving groups can be attached to the furan-2 methyl alcohol using methods similar to those employed to attach toxic leaving groups to the hydroxyl on the C5 propargyl uridine compound, as explained above. A variety of alternative toxic leaving groups are envisioned. In addition, modifications to the length and composition of the R^2 electron conduit component and of the composition of the R^3 spacer element are also envisioned.

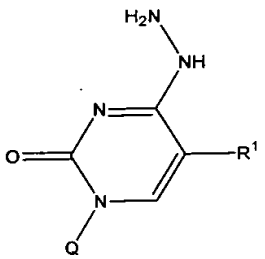
[00144] Compounds based on furano-pyrimidinones can also consist of variously modified "Q" moieties. Compounds can have a free 5' hydroxyl, a 5' monophosphate, or a 5' phosphoramidate group attached to alternative carbohydrate groups. A method for synthesis of such phosphoramidate compounds is accomplished by reacting a 2-deoxy 3'-hydroxy, 5'-hydroxy unprotected nucleotide with a phosphochloridate in the presence of

an HCl scavenger. In one aspect, the phosphochloridate comprises a phosphorus substituent which is derived from an amino acid such as alanine. For example, the phosphochloridate can be phenyl-L-methoxyalanine phosphorochloridate.

C6 Fluoro Uridine and C4 hydrazone based Compounds

[00145] The introduction of fluorine at the C6 position can be synthesized by following the synthetic descriptions of Krajewskas and Shugar (1982), who describe the synthesis of a number of 6 substituted uracil and uridine analogs.

[00146] Chemistry facilitating substitutions at the C4 position of the pyrimidine base are known by those skilled in the art. Examples of literature descriptions include Wallis et al. (1999); Negishi, et al. (1996), Barbato et al. (1991), Barbato, et al. (1989) and Holy et al. (1999). These synthetic techniques also enable combinations of substitutions, for instance at the C4 and C5 positions of the pyrimidine ring (Pluta, et al. 1999) or the C2 and C4 positions of the pyrimidine ring (Zeid, et al. (1999)).

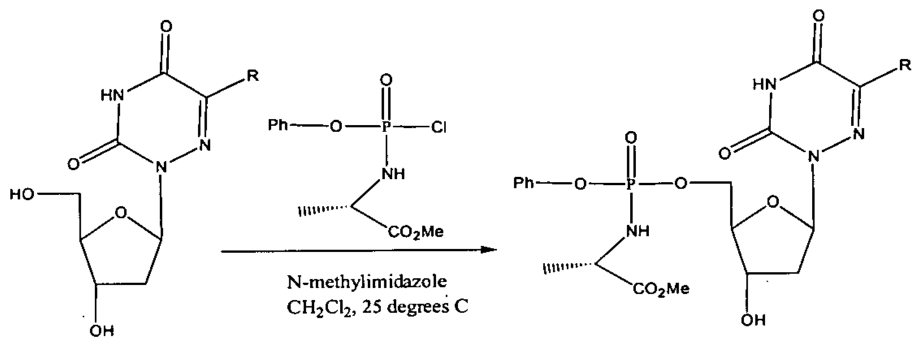


[00147] In another embodiment of the invention, compounds are synthesized by addition of alternative electron conduits, spacer moieties and toxic leaving groups to either the C6 fluoro-uridine base or the C4 hydrazone modified pyrimidine. Methods described above for synthesis of 2-deoxyuridine based compounds can again be employed for the synthesis of such molecules.

Synthesis of Nucleoside Phenyl Methoxyalaninyl Phosphoramidates

[00148] The use of phosphoramidates as phosphate prodrugs for nucleotides was reported by McGuigan, C. et al. (1993) and McGuigan, C. et al. (1994). The phospharamidates were synthesized by reacting 2',3'-dideoxynucleosides with phenyl methoxyalaninyl phosphorochloridate (PMPC).

[00149] Since only one hydroxyl group is present, these reactions usually proceed smoothly. In compounds where more than one hydroxyl group is present, the appropriately protected nucleoside may be required. Since the 5'-OH group of 2'-deoxynucleosides is much less hindered than the 3'-OH group, selective phosphoramidation with PMPC is possible under carefully controlled conditions. Both BVdU and 5FUdR condensed with PMPC in the presence of N-methylimidazole in anhydrous CH_2Cl_2 to give the corresponding phosphoramidates. In both cases, the desired product was readily separable from the starting material using column chromatography on silica gel. The synthetic scheme is summarized below.



[00150] The following synthetic examples are intended to illustrate, but not limit the invention.

Examples 1 and 2

Synthesis of compounds with propargyl tethers

[00151] Using the general synthetic procedure described *supra*, **bis-aziridin-1-yl-phosphinic acid 3-[2-deoxyuridin-5-yl]-prop-2-ynyl ester** was synthesized and analyzed by ^{1}H NMR to yield the following result: ^{1}H NMR ($(CD_3)_2SO$). Salient features: δ 8.28 (d, 1, H6), 6.10 (pseudo-t, 1, H1'), 5.26 (m, exchanges with D_2O, 1, 3'-OH), 5.13 (m, exchanges with D_2O, 1, 5'-OH), 4.81 (q or dd, 2, propargyl-CH_2), 4.24 (m, 1, H3'), 3.57 (m, 2, 5'-CH_2), 2.15-2.0 (m, 8, aziridine-CH_2).

[00152] **Bis-aziridin-1-yl-phosphinothioic acid 3-[2-deoxyuridin-5-yl]-prop-2-ynyl ester** was also synthesized and analyzed by ^{1}H NMR to yield the following result: ^{1}H NMR ($(CD_3)_2SO$). Salient features: δ 8.29 (d, 1, H6), 6.10 (pseudo-t, 1, H1'), 5.22 (m,

exchanges with D_2O, 1, 3'-OH), 5.10 (m, exchanges with D_2O, 1, 5'-OH), 4.88 (q or dd, 2, propargyl-CH_2), 4.31 (m, 1, H3'), 3.52 (m, 2, 5'-CH_2), 2.15-2.0 (m, 8, aziridine-CH_2).

Examples 3 to 8

Synthesis of furano-pyrimidinones

[00153] Using the general synthetic procedure described *supra*, the following compounds were prepared.

Example 3

[00154] 3-(2-Deoxy-β-D-ribofuranosyl)-6-(tetrahydropyran-2-yloxymethyl)furo[2,3-*d*]pyrimidin-2(3H)-one. ^{1}H NMR ($(CD_3)_2SO$) δ 8.80 (s, 1, H4), 6.74 (s, 1, H5), 6.16 (pseudo-t, 1, H1'), 5.27 (d, exchanges with D_2O, 1, 3'-OH), 5.12 (t, exchanges with D_2O, 1, 5'-OH), 4.72 (m, 1, THP-H2), 4.56 (q, 2, CH_2OTHP), 3.92 (m, 1, H4'), 3.64 (m, 2, 5'-CH_2), 2.40 (m, 1, H2'a), 2.03 (m, 1, H2'b), 1.68 and 1.50 (m, 8, THP). Low-resolution mass spectrum (DCI-NH_3) on bis-TMS derivative, *m/z* 323 (B+TMS+H^+), 511 (MH^+), 583 ($M+TMS^+$).

Example 4

[00155] 3-(2-Deoxy-β-D-ribofuranosyl)-6-(hydroxymethyl)furo[2,3-*d*]pyrimidin-2(3H)-one. ^{1}H NMR ($(CD_3)_2SO$) δ 12.0 (bs, 1, OH), 8.24 (s, 1, H4), 6.53 (s, 1, H5), 5.51 (pseudo-t, 1, H1'), 4.42 (m, 2, CH_2OH). Low-resolution mass spectrum (DCI-NH_3), *m/z* 167 (B+$2H^+$), 184 (B+NH_4^+).

Example 5

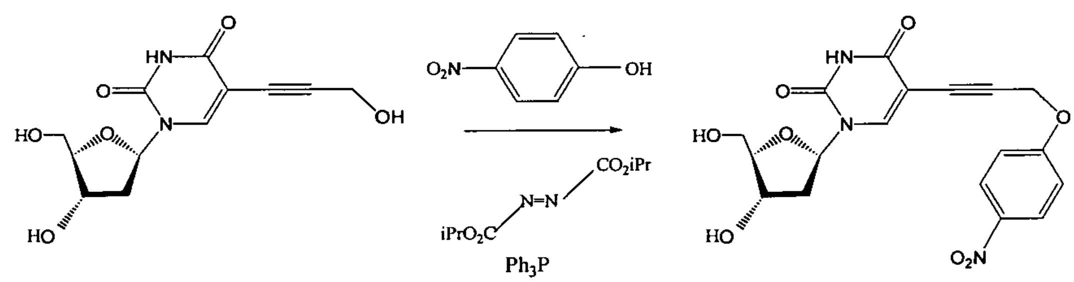
[00156] 1-[6-(Tetrahydropyran-2-yloxymethyl)furo[2,3-*d*]pyrimidin-2(3H)-on-3-yl]-2-deoxy-β-D-ribofuranos-5-yl phenyl methoxy-L-alaninylphosphoramidate. ^{1}HNMR ((CD3)2SO) complicated due to presence of diastereomers. Salient features: δ 8.62 and 8.59 (each s, each 1, H4), 7.4-7.1 (m, 5, PhO), 6.61 and 6.60 (each s, each 1, H5), 6.25 (m, 1, H1'), 4.56 (q, 2, propargyl-CH_2), 3.56 and 3.54 (each s, each 3, CO_2Me), 2.0 (m, 1, H2'b), 1.22 (m, 3, alaninyl-α-Me). Low-resolution mass spectrum (DCI-NH3), *m/z* 167 (B+$2H^+$), 184 (B+H^++NH_4^+-THP).

Example 6

[00157] **1-[6-(Hydroxymethyl)furo[2,3-*d*]pyrimidin-2(3H)-on-3-yl]-2-deoxy-β-D-ribofuranos-5-yl phenyl methoxy-L-alaninylphosphoramidate.** ^{1}H NMR ($CDCl_3$) complicated due to presence of diastereomers. Salient features: δ 8.5 (s, 1, H4), 7.4-7.1 (m, 5, PhO), 6.36 and 6.30 (each s, each 1, H5), 6.23 (m, 1, H1'), 3.67 and 3.65 (each s, each 3, CO_2Me), 2.69 (m, 1, H2'a), 2.10 (m, 1, H2'b), 1.35 (m, 3, alaninyl-α-Me). Low-resolution mass spectrum (DCI-NH_3), *m/z* 525 (MH^+), 595 (MNH_4^+).

Example 7

[00158] The 4-nitrophenyl ether derivative of 5-(3-hydroxy-1-propynyl)-2'-deoxyuridine was prepared according to standard ether synthesis as shown below.



Example 8

5-[3-(4-Nitrophenoxy)-1-propynyl]-2'-deoxyuridine.

[00159] A solution of pre-dried 5-(3-hydroxy-1-propynyl)-2'-deoxyuridine (Robins, M. J. et al. (1983)) (565 mg, 2 mmol) in 40 mL of anhydrous THF under argon was treated with 4-nitrophenol (696 mg, 5 mmol), triphenylphosphine (787 mg, 3 mmol), and diisopropyl azodicarboxylate (590 liters, 3 mmol), and the reaction mixture heated at 60 °C until the solution was clear, and then 1 hour longer. The mixture was allowed to cool to 23 °C and then it was evaporated onto SiO_2 and purified by chromatography using $MeOH/CH_2Cl_2$ as eluent to afford 107 mg (13%) of the desired ether product: melting point 112-118 °C. ^{1}H NMR ($(CD_3)_2SO$) δ 11.65 (s, exchanges with D_2O, 1, NH), 8.29 (s, 1, H6), 8.24 (d, J = 9.3 Hz, 2, *m*-ArH), 7.23 (d, J = 9.3 Hz, 2, *o*-ArH), 6.09 (pseudo-t, 1, H1'), 5.17 (s, 2, propargyl-CH_2), 4.22 (m, 1, H3'), 3.80 (m, 1, H4'), 3.59 (m, 2, 5'-CH_2), 2.13 (pseudo-t, 2,

2'-CH_2). Low-resolution mass spectrum (DCI-NH_3) on *per*-trimethylsilyated material, *m/z* 547 [$M(TMS)_2H^+$], 565 [$M(TMS)_2NH_4^+$], 620 [$M(TMS)_3H^+$].

Example 9

5-(4-Carbethoxy-1,3-butadienyl)-2'-dexoyuridine

[00160] (a) 5-(Carbomethoxyvinyl)-2'-deoxyuridine-3',5'-bis(tetrahydro-2H-pyran-2-yl)ether (I)

[00161] A slurry of 5-(carbomethoxyvinyl)-2'-deoxyuridine (3.0 g, 9.6 mmol), 3,4-dihydro-2*H*-pyran (22 mL, 21.3 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 0.242 g, 0.96 mmol) in dimethylformamide (DMF, 5 mL) was stirred at 50°C for 18 hours. The resulting solution was concentrated *in vacuo* (bath temperature 45°C) to give a thick, pale yellow oil. The oil was dissolved in EtOAc and the solid was filtered. The solution was again concentrated. The oil obtained was purified by column chromatography on silica gel using 50-75% EtOAc/hexane as eluent to give 3.81 g (85%) of pure product as a colorless oil.

(b) 5-(3-Hydroxyprop-1-enyl)-2'-deoxyuridine-3',5'-bis(tetrahydro-2H-pyran-2-yl)ether (II)

[00162] A solution of (I) (3.5 g, 7.27 mmol) in CH_2Cl_2 (14 mL) was cooled to -78°C in a dry ice/acetone bath. Diisobutylaluminum hydride (DIBAL-H) in toluene (1.0 M, 24 mL, 24.0 mmol) was added dropwise over 2 hours while the temperature was maintained at -78°C. The solution was stirred at -78°C for an additional 2 hours and MeOH (2.5 mL) was added dropwise to destroy any excess DIBAL-H. The reaction mixture was cannulated into a mixture of 30% citric acid solution (50 mL), ice (25 g) and EtOAc (30 mL) over ca. 20 minutes. The phases were separated and the aqueous phase was extracted with EtOAc (2 × 25 mL). The combined organic phase was washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated to give 3.288 g (100%) of colorless oil

(c) 5-(3-Oxoprop-1-enyl)-2'-dexoyuridine-3',5'-bis(tetrahydro-2H-pyran-2-yl)ether (III)

[00163] To a solution of crude (II) obtained from above (1.988 g, 4.4 mmol) in CH_2Cl_2 (9 mL) was added solid pyridinium dichromate (PDC; 1.82 g, 4.8 mmol) with water cooling.

The suspension was stirred while acetic acid (0.4 mL) was added dropwise. The water bath was removed and the reaction was stirred at room temperature for 1 hour. The crude product was filtered through a pad of florisil (2 × 2.5 cm) and the florisil washed with 35 mL EtOAc. The brown solution obtained was filtered through another column of florisil (3.5 cm diam × 2.5 cm height). The filtrate was concentrated to give 1.273 g (64% yield) of very light brown oil.

(d) 5-(4-Carbethoxy-1,3-butadienyl)-2'-dexoyuridine-3',5'-bis(tetrahydro-2H-pyran-2-yl)ether (IV)

[00164] (Carbethoxymethylene)triphenylphosphorane (0.32 mg, 0.92 mmol) was added to a solution of the crude aldehyde (III) (0.344 g, 0.77 mmol). The solution darkened and turned rust color. After 1 hour, (III) was completely consumed as judged by thin layer chromatography. The solvent was evaporated and the crude product was purified by column chromatography on silica gel using 35-45% EtOAc/hexane as eluent. The pure product (0.310 g, 78% yield) was obtained as colorless oil.

(e) 5-(4-Carbethoxy-1,3-butadienyl)-2'-dexoyuridine (V)

[00165] 5-(4-Carbethoxy-1,3-butadienyl)-2'-dexoyuridine-3',5'-bis(tetrahydro-2H-pyran-2-yl)ether (IV) (0.637 g, 1.22 mmol) was dissolved in MeOH (1.5 mL) and PPTS (0.049 g, 0.16 mmol) was added. The solution was stirred at 50°C for 7.5 hours and left at room temperature overnight. A white precipitate was formed. The reaction mixture was cooled to 0°C and filtered to give pure (V) as a white solid (0.188 g). The filtrate was concentrated and chromatographed on silica gel using 50-100% EtOAc/hexane as eluent to give a further 0.180 g product. The total yield of the product was 0.368 g (86%).

[00166] ^{1}H NMR (DMSO-d_6): 1.22 (3H, t, J = 7 Hz), 2.17 (2H, br t, J = 5.5 Hz), 3.55-3.75 (2H, m), 3.81 (1H, m), 4.12 (2H, q, J = 7 Hz), 4.25-4.28 (1H, m), 5.19 (1H, t, J = 4.8 Hz), 5.27 (1H, d, J = 4.1 Hz), 5.98 (1H, d, J = 14.5 Hz), 6.14 (1H, t, J = 6.3 Hz), 6.75 (1H, d, J = 14.5 Hz), 7.18-7.30 (2H, m), 8.30 (1H, s), 11.56 (1H, s).

Example 10

5-(4-Carbomethoxy-1,3-butadienyl)-2'-dexoyuridine (Va)

[00167] A solution of triethylamine (3.9 mL, 28.2 mmol) in dioxane (12 mL) was deareated by bubbling nitrogen through for 15 minutes. Palladium acetate (0.60 g, 0.26

mmol) and triphenylphosphine (0.183 g, 0.70 mmol) were added and the solution was heated at 70°C for 20 minutes to give a dark brown solution. 5-Iodo-3'-deoxyuridine (5.0 g, 14.1 mmol) and methyl 2,4-pentadienoate (2.5 g, 22.3 mmol) were added and the mixture was heated under reflux for 15 hours. The solvent and volatile components were evaporated in vacuo and the residue was partitioned between water (15 mL) and EtOAc (15 mL). The phases were separated and the aqueous phase was extracted twice with EtOAc (10 mL each). The combined organic phase was washed with brine and concentrated. The residue was dissolved in MeOH (15 mL) and allowed to cool to room temperature. The solid formed was collected by filtration, washed with a small quantity of MeOH and dried in vacuo to give 0.38 g brown powder.

[00168] ^{1}H NMR (DMSO-d_6): 2.17 (2H, t, J = 6.4 Hz), 3.55-3.70 (2H, m), 3.66 (3H, s), 3.82 (1H, q, J = 3.6 Hz), 4.27 (1H, m), 5.18 (1H, t, J = 4.9 Hz), 5.26 (1H, d, J = 4.5 Hz), 5.99 (1H, d, J = 14.4 Hz), 6.14 (1H, d, J = 6.4 Hz), 6.74 (1H, d, J = 14.8 Hz), 7.20-7.35 (2H, m), 8.30 (1H, s), 11.56 (1H, s).

[00169] The filtrate from above was concentrated and chromatographed on silica gel using 60-100% EtOAc/hexanes as eluent to give another 0.70 g of product as a brown foam. The combined yield was 1.08 g (22.6%).

Example 11

5-(4-Carboxy-1,3-butadienyl)-2'-dexoyuridine (VI)

Method I

[00170] 5-(4-Carbethoxy-1,3-butadienyl)-2'-dexoyuridine (V, from Example 9) (0.449 g, 1.28 mmol) was dissolved in 2N NaOH (3 mL) and stirred at 25°C. After 20 minutes, a precipitate was formed and TLC showed that the starting material was completely consumed. The mixture was cooled to 0°C and acidified to pH 1 with 2N HCl. The resulting off-white solid was filtered off, washed with water and dried *in vacuo* to give 0.225 g (54%) product.

[00171] ^{1}H NMR (DMSO-d_6): 2.12-2.19 (2H, m), 3.50-3.70 (2H, m), 3.75-3.85 (1H, m), 4.24-4.29 (1H, m), 5.19 (1H, t, J = 4.8 Hz), 5.27 (1H, d, J = 4.2 Hz), 5.80-5.95 (1H, m), 6.14 (1H, t, J = 6.4 Hz), 6.60-6.75 (1H, m), 7.15-7.25 (2H, m), 8.26 (1H, s), 11.56 (1H, s), 12.16 (1H, br s).

[00172] The filtrate and washings were combined and evaporated to dryness. The resulting sticky yellow solid was dissolved in MeOH from which a white precipitate was formed. The solid was filtered off to give an additional 0.200 g of product.

Method II

[00173] The title compound can also be prepared from 5-(4-carbomethoxy-1,3-butadienyl)-2'-dexoyuridine (Va, from Example 10) in comparable yield as mentioned above.

Example 12

5-(4-Bromo-1*E*,3*E*-butadienyl)-2'-dexoyuridine (VIIa) and 5-(4-Bromo-1*E*,3*Z*-butadienyl)-2'-dexoyuridine (VIIb)

[00174] To a solution of 5-(4-carboxy-1,3-butadienyl)-2'-dexoyuridine (VI) (0.200 g, 0.62 mmol) in DMF (1 mL) was added $KHCO_3$ (0.185 g, 1.84 mmol) and the mixture was stirred for 20 minutes at 25°C. A solution of *N*-bromosuccinimide (0.117 g, 0.65 mmol) in DMF (0.3 mL) was added dropwise. Smooth gas evolution (CO_2) occurred throughout the addition. The resulting brown suspension was stirred for 2 hours at 25°C at which time TLC showed that **(VI)** was completely consumed. Water (10 mL) was added to the suspension and the resulting solution was extracted with EtOAc (2 × 15 mL). The extract was dried over $MgSO_4$ and the solvent was evaporated *in vacuo* to give a yellow solid (178 mg, 80% yield) consisting of a mixture of two isomers as shown by ^{1}H NMR. The crude product was separated by semi-preparative HPLC (reversed phase C18 column) using 20% acetonitrile in water as the mobile phase to give the following isomers:

[00175] **5-(4-Bromo-1*E*,3*Z*-butadienyl)-2'-dexoyuridine:** retention time 10.5 minutes; ^{1}H NMR: (DMSO-d_6): 2.11-2.18 (2H, m), 3.50-3.70 (2H, m), 3.80 (1H, distorted q, J = 3.5 Hz), 4.25 (1H, br s), 5.08 (1H, br s), 5.25 (1H, br s), 6.15 (1H, t, J = 6.5 Hz), 6.40 (1H, d, J = 7 Hz), 6.53 (1H, d, J = 15.6 Hz), 6.83 (1H, dd, J = 7, 10 Hz), 7.39 (1H, dd, J = 10, 15.6 Hz).

[00176] **5-(4-Bromo-1*E*,3*E*-butadienyl)-2'-dexoyuridine**: retention time 15.1 minutes; ^{1}H NMR (DMSO-d_6): 2.12-2.16 (2H, m), 3.50-3.70 (2H, m), 3.80 (1H, q, J = 3.2 Hz), 4.26 (1H, m), 5.13 (1H, br s), 5.25 (1H, br s), 6.14 (1H, t, J = 6.5 Hz), 6.36 (1H, d, J = 15.6 Hz), 6.67 (1H, d, J = 13.1 Hz), 6.84 (1H, dd, J = 11, 13.1 Hz), 7.04 (1H, dd, J = 11, 15.6 Hz).

Example 13

[00177] Using the procedures mentioned in Example 11, Method II, the following compounds can be obtained in a similar fashion: 5-(4-chloro-1,3-butadienyl)-2'-dexoyuridine (using *N*-chlorosuccinimide in place of *N*-bromosuccinimide in Step B); 5-(4-iodo-1,3-butadienyl)-2'-dexoyuridine (using iodine in sodium idodide in place of *N*-bromosuccinimide).

Example 14

Phenyl *N*-methoxy-*L*-alaninyl phosphorochloridate

[00178] *L*-alanine methyl ester hydrochloride (245.8 g; 1.76 mol) was placed in a 12 liter three-neck round bottom flask (equipped with a mechanical stirrer and thermometer) followed by 4.0 liters of dichloromethane. The mixture was stirred for 15 minutes at room temperature. Phenyl phosphodichloridate (370.0 g; 1.76 mol) was added to the mixture and stirring was continued for 15 minutes at room temperature. The flask was placed in the bath with dry ice and the stirring was continued for 20 minutes until a uniform suspension was formed.

[00179] Freshly distilled tri-n-butylamine (626.5 g; 3.38 mol) was added dropwise (~90 minutes) with vigorous stirring to the reaction mixture so that the temperature inside the flask was held at ~0°C. The bath was removed and the stirring was continued for 6 hours at room temperature. The solution was concentrated to ~2.84 liters by evaporating several portions of the mixture on a rotary evaporator and the mixture was sealed under argon and stored at -20°C. The product was 85% pure by phosphorus NMR to give an estimated concentration of phenylmethoxyalaninyl phosphochloridate of ~0.5 M.

Example 15

5-(2-Bromovinyl)-2'-deoxyuridine phenyl *N*-methoxy-*L*-alaninyl phosphoramidate (NB1011)

[00180] The reaction was performed under argon atmosphere. 5-(2-bromovinyl)-2'-deoxyuridine (BVdU) (204 g; 612 mmol) was placed in three-neck 3 liter round bottom flask equipped with mechanical stirrer. The flask was placed in ice-water bath and 1600 mL (~ 800 mmol) of phenylmethoxyalaninyl phosphochloridate reagent were added using an addition funnel over 15 minutes with vigorous stirring of the reaction mixture, followed

by the addition of 100 mL of N-methylimidazole over 5 minutes using syringe. After 5 minutes the mixture became clear and after 10 minutes the ice-water bath was removed to allow the mixture to warm up to room temperature while stirring was continued. The reaction was monitored by reversed phase HPLC and was complete in 3 hours. The reaction was quenched by the addition of 100 mL of methanol and the mixture was evaporated to an oil, re-dissolved in 6 liters of dichloromethane and passed through 800 g of silica gel. The major portion of BVdU-PA, referred to herein as NB1011, was passed through the column during the loading and finally the elution of NB1011 was completed by passing 5 liters of 5% methanol in dichloromethane. All fractions containing NB1011 were combined and evaporated to an oil, the residue was dissolved in 4 liters of ethyl acetate and the mixture was extracted with water (2 x 2 liters). The organic layer was dried with sodium sulfate, filtered, and washed with ethyl acetate (3 x 300 mL). The combined filtrate and washings were evaporated to produce a lightly colored white foam; total weight ~540 g.

[00181] The crude product was purified by two silica gel chromatography using 0-5% MeOH in CH_2Cl_2 and 10% MeOH in CH_2Cl_2, respectively, as eluent. The yield of product (>98% pure) was 64 g.

Example 16

[00182] Using the methods described in Example 15, the phenyl *N*-methoxy-*L*-alanyl phosphoramidates of the following nucleosides were prepared:

[00183] 5-(4,4-dibromo-1,3-butadienyl)-2'-deoxyuridine;

[00184] 5-(2-chlorovinyl)-2'-deoxyuridine;

[00185] 5-trifluoromethyl-2'-deoxyuridine;

[00186] 5-(4-carbethoxy-1,3-butadienyl)-2'-deoxyuridine;

[00187] 5-(4-carbomethoxy-1,3-butadienyl)-2'-dexoyuridine;

[00188] 5-(4-bromo-1E,3E-butadienyl)-2'-deoxyuridine;

[00189] 5-(4-bromo-1E,3Z-butadienyl)-2'-deoxyuridine;

[00190] 5-(trimethylsilylethynyl)-2'-deoxyuridine;

[00191] 5-(ethynyl)-2'-deoxyuridine;

[00192] 5-(1-decynyl)-2'-deoxyuridine;

[00193] 3-(2'-deoxy-□-D-ribofuranosyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one; and

[00194] 3-(2'-deoxy-□-D-ribofuranosyl)-6-octyl-2,3-dihydrofuro[2,3-d] pyrimidin-2-one.

[00195] Chemical assays for products, for example, where a reaction product is an anti-metabolite of the bromovinyl-derivatives of dUMP, are described in the Examples provided below or by Barr, P. J. et al. (1983).

Example 17

Induction and Assessment of Arthritis

[00196] Arthritis was induced in male DBA/1 mice (8-10 weeks old) by intradermal injection of bovine type II collagen, purified in-house at the Kennedy Institute of Rheumatology as previously described (Miller, E. J. et al. (1972)). Collagen was administered in complete Freund's adjuvant (Difco, Detroit, Michigan). Onset of arthritis was variable, occurring from Day 14 up to Day 40 after immunization. Arthritis onset was considered to occur on the day that swelling and/or erythema were observed. Clinical score is a composite of disease severity and the number of limbs affected, and was monitored daily from onset of disease and used as an assessment of disease progress. The scoring used was: 0, Normal; 1, slight swelling with erythema; 2, pronounced swelling; 3, joint rigidity. In addition, the extent of paw swelling reflects the degree of edema in affected limbs. Arthritis increased progressively over 10 days as reflected by both clinical score and paw swelling.

Example 18

Treatment of Animals with Anti-TNF or NB 1011

[00197] Anti-TNF antibody was used in these experiments was as described by Marinova-Mutafchieva, L. et al. (2000). NB1011 was administered daily by intraperitoneal administration at 2.5 mg total dose per day. Anti-TNF antibody was compared with NB1011 because, at present, antiTNF antibody is the optimal single agent for treatment of collagen induced arthritis (Marinova-Mutafchieva, L. et al. (2000)).

[00198] Success in this model has been shown to be predictive for clinical success in the development of new agents to treat inflammatory disease, especially rheumatoid arthritis (Elliott et al. (1994); Feldmann et al. (1998)). This model therefore represents an ideal setting for establishing proof of concept for new agents to treat rheumatoid arthritis, and potentially other autoimmune and inflammatory diseases.

[00199] Following immunization with collagen, mice were maintained until a significant clinical score for disease progression was achieved (between 2.5 and 3.5, see Figure 1 and Methods). Mice were then treated with control saline injections, NB1011, or with anti-TNF antibody as a positive control. The results (Figure 1) show that the NB1011-treated group exhibited significant disease suppression ($p < 0.05$), similar to the anti-TNF control, when compared with the saline-treated control group. There was no significant difference between the NB1011 and anti-TNF groups with regard to clinical score. Paw swelling is an alternative measure of CIA disease severity. When paw swelling was used as a criteria for disease suppression, comparable results were observed (Figure 2). In this second measure of efficacy, both the NB1011 and anti-TNF groups demonstrated significant disease suppression as compared to the saline-treated control group ($p < 0.05$). Again, there was no significant difference between the NB1011 and anti-TNF groups, although suppression of swelling may have been less dramatic with NB1011. A further significant outcome of this work is that by comparison with earlier reported work, NB1011 appears to have activity superior to anti-angiogenesis agents, an anti-CD4 immunosuppressive agent, and cannabidiol, a third experimental agent currently being considered for use to treat rheumatoid arthritis, and potentially other autoimmune and inflammatory disorders (Malfait, A. M. et al. (2000); Miotla, J. et al. (2000); Marinova-Mutafchieva, L. et al. (2000)).

[00200] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

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